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AMYOTROPHIC LATERAL SCLEROSIS

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Introduction

Welcome to the 21st International Symposium on ALS/MND, organized by the Motor Neurone Disease Association, UK in collaboration with the International Alliance of ALS/MND Associations. This year we return to the USA as our hosts at the ALS Association mark their 25th year in the fight against the disease.

The Symposium Programme Committee, chaired for the first time this year by Prof Wim Robberecht, has compiled a stimulating platform programme that includes ground breaking scientific topics and thought provoking clinical sessions. From opening plenary talks that cover new perspectives in our understanding of the disease and an examination of practice guidelines, the parallel scientific and clinical sessions go on to explore a range of pertinent themes including RNA biology, newly generated disease models, holistic care and care education and practice. A session on glial cell biology emphasizes the importance of 'thinking outside the neuron' whilst a surrogate markers session includes novel suggestions for measuring disease progression.

This year the committee has been pleased to reflect the quality and quantity of the poster presentations by including two poster sessions, allowing for more extensive discussion and hopefully encouraging some lively debate.

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SESSION 1 JOINT OPENING SESSION

C1 NEW PERSPECTIVE ON AMYOTROPHIC LATERAL SCLEROSIS AS TDP-43 PROTEINOPATHIES

LEE V M-Y

Center for Neurodegenerative Disease Research, University of Pennsylvania, Philadelphia, PA, United States

E-mail address for correspondence: vmylee@upenn.edu

Keywords: TDP-43, degeneration, neurons

The disease protein in frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U), now referred to as FTLD-TDP, and amyotrophic lateral sclerosis (ALS) was identified recently as the TAR DNA-binding protein (TDP-43) thereby providing a molecular link between these two disorders. Moreover, the discovery of mutations in the TDP-43 gene (*TARDBP)* in familial ALS and FTLD-U with similar TDP-43 pathology suggests that it is a primary cause of these disorders. Although TDP-43 is normally a nuclear protein, cytoplasmic accumulations of pathological TDP-43 as well as nuclear clearance of endogenous TDP-43 are major neuropathology found in TDP-43 proteinopathies including ALS and other motor neuron diseases. This disease feature was recapitulated in cell models that accumulate cytoplasmic TDP-43 inclusions with concomitant clearance of nuclear TDP-43, suggesting both gain and loss of functions are potential mechanisms of disease pathogenesis. We next generated transgenic (Tg) mice conditionally expressing human wild type TDP-43 (hTDP-43-WT) and hTDP-43 with a defective nuclear localization signal (hTDP-43-ΔNLS) directed by the CaMKIIα promoter to elucidate mechanisms of neurodegeneration in TDP-43 proteinopathies. Although expression of hTDP-43 WT or hTDP-43-ΔNLS led to the formation of rare phosphorylated and ubiquitinated TDP-43 inclusions, significant neuron loss in a time dependent manner was observed in selectively vulnerable forebrain regions. This was accompanied by corticospinal tract degeneration and motor spasticity which taken together, recapitulates key aspects of FTLD and primary lateral sclerosis. Remarkably, neurodegeneration was linked to a dramatic downregulation of endogenous mouse TDP-43 in nuclei of affected neurons associated with changes in gene expression, especially up regulation of genes involved in chromatin assembly. Our data suggest that perturbation of highly regulated endogenous nuclear TDP-43 results in loss of functions and changes in downstream gene regulatory pathways that trigger degeneration of selectively vulnerable neurons.

C2 AAN PRACTICE PARAMETERS: WHAT THEY TELL US AND WHAT THEY DON'T

MILLER RG[1], JACKSON CE[2], KASARSKIS EJ[3], ENGLAND JD[4], FORSHEW DA[1], JOHNSTON WS[5], KALRA S[5], KATZ JS[1], MITSUMOTO H[6], ROSENFELD J[7], SHOESMITH C[8], STRONG MJ[8], WOOLLEY SC[1], THE ALS PRACTICE PARAMETER TASK FORCE AND THE QUALITY STANDARDS SUBCOMMITTEE OF THE AMERICAN ACADEMY OF NEUROLOGY[1]

[1]*California Pacific Medical Center, San Francisco, CA, United States,* [2]*University of Texas Health Science Center, San Antonio, TX, United States,* [3]*University of Kentucky and VA Medical Centers, Lexington, KY, United States,* [4]*LSUHSC School of Medicine, New Orleans, LA, United States,* [5]*University of Alberta, Edmonton, AB, Canada,* [6]*Columbia University, New York, NY, United States,* [7]*UCSF-Fresno Central California Neuroscience Institute, Fresno, CA, United States,* [8]*University of Western Ontario, London, ON, Canada*

E-mail address for correspondence: millerrx@sutterhealth.org

Keywords: patient management, practice parameters, guidelines

Background: The American Academy of Neurology (AAN) issued evidence-based practice parameters for managing patients with ALS/MND in 2009.

Objective: To critically review the value and limitations of the AAN guidelines.

Methods: The authors identified 10 Class I studies, 13 Class II studies, and 73 Class III studies for managing ALS.

Results: Evidence-based recommendations include the use of riluzole to slow disease progression, but cost-benefit analyses for this therapy are needed, and the literature failed to identify other effective disease-modifying agents. Noninvasive ventilation lengthens survival and improves quality of life, yet there was no evidence regarding the most sensitive tests of respiratory function in ALS or the optimal time to initiate noninvasive ventilation. The literature also lacked evidence regarding the most reliable measure of respiratory insufficiency in bulbar patients, and did not adequately address strategies for decision making regarding tracheostomy.
Percutaneous endoscopic gastrostomy (PEG) or radiologically inserted gastrostomy (RIG) can stabilize weight and prolong survival. While nutrition has relevance, questions remain regarding when to initiate PEG or RIG and how it impacts quality of life and survival. Additionally, topics including dietary management (eg high calorie or high fat diet, blood lipids and statins), the value of PEG/RIG in patients without dysphagia, and the effect of vitamins and supplements all deserve further study.
Multidisciplinary clinic referral can optimize health care delivery, and correlates with prolonged survival and enhanced quality of life. While this new recommendation is pertinent, cost effectiveness studies of multidisciplinary clinics are needed.

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Refractory sialorrhea is improved by botulinum toxin B, and pseudobulbar affect responds to dextromethorphan with quinidine. However, optimal medical therapy for each intervention is not clear. Other symptoms have not been systematically studied (eg cramps, spasticity, fatigue, insomnia, laryngospasm, constipation, anxiety, depression, pain and terminal dyspnea). Nonetheless, clinical experience suggests symptomatic treatments that appear helpful until more evidence is available to guide management.

While cognitive and behavioral screening was recommended, the literature failed to clarify the impact of frontotemporal impairment on compliance and survival, and there were no trials evaluating pharmacologic intervention for cognitive impairment in ALS.

Comment: While we are waiting for the cure, there are treatments available for patients with ALS that alleviate suffering. Noninvasive ventilation, PEG/RIG, riluzole, and multidisciplinary clinics are the most important and have the best evidence. More high-quality, controlled studies are needed to guide management and assess outcomes in patients with ALS. These may address the domains reviewed in the practice parameters, as well as clinically important issues not addressed such as the role of gene testing, equipment for mobility, the role of exercise, and management of end of life issues.

SESSION 2A RNA BIOLOGY IN ALS

C3 USING EMBRYONIC STEM CELLS TO STUDY MOTOR NEURON/GLIA INTERACTIONS IN ALS

PHATNANI HP[1], FRIEDMAN BA[1], CARRASCO MA[1], PAULI F[2], REDDY T[2], MURATET M[2], MYERS R[2], MANIATIS T[1]

[1]*Columbia University Medical Center, Department of Biochemistry and Molecular Biophysics, New York, NY, United States,* [2]*HudsonAlpha Institute for Biotechnology, Huntsville, AL, United States*

E-mail address for correspondence: tm2472@mail.cumc.columbia.edu

Keywords: glia, motor neurons

Amyotrophic Lateral Sclerosis (ALS) is a result of the selective dysfunction and progressive degeneration of motor neurons. Although the underlying disease mechanisms remain unknown, recent *in vivo* and cell culture studies have implicated glial cells in motor neuron degeneration in ALS.
We have made use of the SOD1 mouse model of ALS to study the effect of glial cells bearing the mutant SOD1 transgene on motor neuron viability in cell culture. Specifically, we have studied the gene expression profiles of co-cultured mouse embryonic stem (ES) cell derived motor neurons and primary glia using the Illumina deep sequencing platform (RNAseq). In this study, we vary both the genotype of the motor neurons and glia, as well as time in culture as a means of examining both cell autonomous and non-cell autonomous effects of the mutant transgene. In addition, we carry out parallel studies with spinal cord samples from mutant and wildtype SOD1 mice, and compare both the *in vivo* and *in vitro* derived data sets with laser capture microdissection studies of both the ALS mouse model and human ALS patient samples.
We have detected significant cell autonomous and non-autonomous changes in gene expression in both motor neurons and glia, indicating that the two cell types profoundly affect each other's gene expression. In addition, we find a remarkable concordance between the different data sets mentioned above, thus validating the *in vitro* approach. We are currently analyzing these data sets to identify changes in the expression of specific genes and signalling pathways that may contribute to motor neuron degeneration in ALS.

C4 ROLE OF RNA PROCESSING IN THE PATHOGENESIS OF AMYOTROPHIC LATERAL SCLEROSIS

LAGIER-TOURENNE C[1,2], POLYMENIDOU M[1,2], HUTT KR[2], LIANG TY[2], HUELGA S[2], LING S[1,2], MAZUR C[3], WANCEWICZ E[3], BENNETT FC[3], YEO GW[2], CLEVELAND DW[1,2]

[1]*Ludwig Institute for Cancer Research, La Jolla, California, United States,* [2]*Cellular and Molecular Medicine, University of California San Diego, La Jolla, California, United States,* [3]*Isis Pharmaceuticals, Carlsbad, California, United States*

E-mail address for correspondence: clagiert@ucsd.edu

Keywords: RNA processing, TDP-43, high-throughput sequencing

Background: The identification of Amyotrophic Lateral Sclerosis (ALS)-causing mutations in TDP-43 and FUS/TLS, two RNA/DNA binding proteins, combined with TDP-43 mislocalization in most incidences of sporadic ALS, suggests that altered RNA processing may play an important role in the pathogenesis of ALS. A fundamental issue is the precise role(s) of TDP-43 in RNA metabolism regulation and how alterations in its properties may underlie neurodegeneration. TDP-43 has been proposed to participate in several steps of RNA processing including alternative splicing regulation. Few RNA targets of TDP-43 have been identified and a comprehensive protein-RNA interaction map still needs to be defined.

Objectives: To identify *in vivo* RNA targets of TDP-43 and validate the roles of TDP-43 in the processing of these targets.

Methods: We have used cross-linking immunoprecipitation coupled with high-throughput sequencing (HITS-CLIP or CLIP-seq) to identify RNAs bound by TDP-43 in mouse brain. We have subsequently determined the effects of TDP-43 loss of function on RNA expression and splicing patterns by using high-throughput sequencing of cDNA (RNA-seq).

Results: Greater than 2 million uniquely mapped reads enabled the accurate generation of clusters using gene-specific thresholds to define TDP-43 binding sites. Consistent with a proposed role of TDP-43 as a splicing regulator, multiple binding sites proximal to alternatively spliced exons were identified. To validate the role of TDP-43 in the regulation of alternative splicing via these sites, downregulation of TDP-43 *in vivo* was achieved in an otherwise normal adult mouse brain using direct injection of antisense oligonucleotides against TDP-43. Transcriptome profiling from brains with TDP-43 knockdown to 80% of endogenous levels confirmed its roles in alternative splicing and gene expression regulation.

Discussion and conclusions: Genome-wide identification of validated RNA targets is a first step in the elucidation of the molecular mechanisms underlying death of motor neurons in ALS. This study reinforces the crucial role of splicing regulation for neuronal integrity and potentially identifies candidate genes whose altered processing is central to ALS pathogenesis.

C5 GENETIC AND BIOCHEMICAL ANALYSIS OF TDP-43 PROTEINOPATHY

KIM SH, HANSON K, TIBBETTS R

University of Wisconsin, Madison, WI, United States

E-mail address for correspondence: rstibbetts@wisc.edu

Keywords: TDP-43, FUS/TLS, motor neuron

It was recently found that dominant mutations in two related RNA-binding proteins, TDP-43 (43 kDa TAR DNA-binding domain protein) and FUS/TLS (fused in sarcoma/translated in liposarcoma) cause a subset of ALS. The convergent ALS phenotypes associated TDP-43 and FUS/TLS mutations are suggestive of a functional relationship; however, whether or not TDP-43 and FUS/TLS operate in common pathways is not known. We have employed biochemical and genetic approaches to discover pathways controlled by TDP-43 and FUS/TLS in mammalian neurons and in the fruit fly, *Drosophila melanogaster*. We have found that TDP-43 and FUS/TLS directly interact to form a complex at endogenous expression levels in mammalian cells. Binding was mediated by an unstructured TDP-43 C-terminal domain and occurred within the context of a 300-400 kDa complex that also contained C-terminal cleavage products of TDP-43 linked to neuropathology. On the other

hand, FUS/TLS and TDP-43 C-terminal fragments were excluded from large molecular mass TDP-43 ribonucleoprotein complexes that also contain the nuclear polyA-binding protein, PABP2. TDP-43 and FUS/TLS collaborate to regulate mRNA stability of HDAC6, and likely other mRNA targets, in mammalian cells, suggesting that these two ALS-associated proteins participate in common biochemical processes. We have further explored the mechanisms of TDP-43-dependent neurodegeneration in *Drosophila*, where expression of mutant or wild-type human TDP-43 proteins in motor neurons causes age-dependent paralysis and lethality. We are using the *Drosophila* ALS model to discover neuron-selective gene expression changes occurring before, during, and after onset of paralysis. The goal of these studies is to identify genes and small molecules that modify TDP-43-associated motor neuron death and to apply these findings to mammalian preclinical models. We will discuss our initial findings implicating two pathways as potentially key determinants in TDP-43-dependent neurodegeneration *in vivo*.

C6 CHARACTERIZING THE ROLE OF TDP-43 IN ALS

FREIBAUM B, SMITH R, ALAMI N, TAYLOR PJ

St. Jude Children's Research Hospital, Memphis, TN, United States

E-mail address for correspondence: brian.freibaum@stjude.org

Keywords: TDP-43, RNA metabolism, proteomics

Background: The TAR DNA-binding protein 43 (TDP-43) is the major disease protein in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin inclusions (FTLD-U), defining a novel class of neurodegenerative conditions: *the TDP-43 proteinopathies*. TDP-43 is a highly conserved nuclear heterogeneous ribonucleoprotein (hnRNP) that has been implicated in regulating the transcription, splicing and translation of target genes. In the brain, TDP-43 is normally found in the nuclei of neurons and some glial cells, although dynamic studies performed *in vitro* have shown TDP-43 to shuttle between the nucleus and cytoplasm. In TDP-43 proteinopathies, TDP-43 is frequently found redistributed from the nucleus to insoluble aggregates in the cytoplasm, although the significance of this finding is unknown. Mutations in the gene encoding TDP-43 have been identified in association with sporadic and familial forms of ALS, strongly implicating this protein as contributing to pathogenesis in these diseases.

Objectives: In this project, we seek to define the role of TDP-43 in disease pathogenesis using biochemical and molecular genetic approaches in both *in vitro* and *in vivo* model systems.

Methods: We characterized the TDP-43 interactome using proteomics in human tissue culture cells and developed transgenic Drosophila expressing wild type and numerous mutant forms of TDP-43. The mutant forms of TDP-43 we have evaluated are designed to interrogate the contribution of distinct TDP-43 domains to pathogenesis.

Results: Global proteomic analysis reveals that TDP-43 interacts with two distinct protein interaction networks, each involved in different aspects of RNA metabolism. The first is a group of predominantly nuclear proteins that regulate RNA splicing; the second is a group of predominantly cytoplasmic proteins involved in mRNA translation. Many of these interactions were found to be dependant on the RNA binding domain of TDP-43. Additionally, TDP-43 was found to interact with components of RNA granules and is recruited to RNA granules in an RNA dependant manner. To date, we have not identified any altered interactions in disease causing TDP-43 mutants.
In vivo studies using transgenic *Drosophila* demonstrate motor neuron degeneration in response to TDP-43 expression. This phenotype is enhanced by the M337V mutation associated with familial ALS, or by mutations designed to promote cytoplasmic localization of TDP-43.

Discussion and conclusion: Based on the results of these studies, we hypothesize that altered RNA metabolism plays a key role in the pathogenesis of ALS. Our proteomic analysis of TDP-43 interacting proteins reveals strong association with RNA binding proteins in support of this hypothesis. Ongoing work is being done to characterize the TDP-43 interactome in motor neurons as well as identifying additional genes that are required for ALS pathogenesis.

C7 RNA TARGETS OF TDP-43 IDENTIFIED USING UV-CLIP ARE DEREGULATED IN ALS

XIAO S, SANELLI T, ZINMAN L, ROBERTSON J

University of Toronto, Toronto, Canada

E-mail address for correspondence: jan.robertson@utoronto.ca

Keywords: TDP-43, CLIP, RNA

Background: Tar DNA Binding protein-43 (TDP-43) is a major component of the pathological inclusions characteristic of Amyotrophic Lateral Sclerosis (ALS) and mutations in the TDP-43 gene are causative of about 4% of fALS cases and ~1% of sALS cases. TDP-43 plays important roles in RNA processing and regulation, including transcriptional repression, mRNA stability and alternative splicing. Based on this, it is therefore likely that abnormalities of TDP-43 in ALS will be reflected in defects of RNA processing.

Objectives: Our first objective was to identify the RNA targets of TDP-43. Our second objective was to determine if any of the identified RNA targets are disrupted in ALS.

Methods: RNA targets of TDP-43 were identified using UV-CLIP (cross linking and immunoprecipitation) applied to the human neuroblastoma cell line, SHSY5Y.
In this method, UV-treatment of SHSY5Y cells covalently crosslinks TDP-43 to its target RNAs. The TDP-43-RNA complexes are then immunoprecipitated with polyclonal TDP-43 antibody, treated with RNAse, radiolabeled with γ-^{32}P and resolved by SDS-PAGE. The TDP-43-RNA complexes are excised from the gel and the bound RNA tags (CLIP-tags) released with proteinase k, cloned and sequenced. The sequences are then BLASTed to identify the corresponding genes.

Results: Conventional cloning and sequencing was used to validate the UV-CLIP technique, as high throughput sequencing generates an overwhelming abundance of data that can be difficult to process. After TA-cloning of the CLIP-tags, 250 clones were empirically selected for analyses. Of these, after numerous quality control steps, 101 genes were identified as RNA binding targets of TDP-43. These genes represented multiple biological pathways. The TDP-43 binding sites were predominantly within intronic sequences (77%), but also included 3'UTRs (4%), noncoding RNAs (5%) and intergenic regions (14%). Importantly the two most common recognition motifs were TG-rich (49%) and polypyrimide-rich (17.65%) sequences, as has been previously reported by others, validating our methodology for isolating bona fide TDP-43 RNA targets. RT-PCR of spinal cord mRNA was used determine if candidate genes were deregulated in ALS. The strategy for primer design was based on the location

of TDP-43 binding and from web-based predictions as to whether or not the adjacent exons were alternatively spliced. Using this approach we show that 5 of the genes identified by TDP-43-CLIP are abnormally spliced in ALS versus controls.

Discussion: We have used UV-CLIP to identify RNA targets of TDP-43 and shown that 5 of the identified genes are deregulated in ALS versus controls. This supports the hypothesis that abnormalities of TDP-43 in ALS cause defects in RNA processing.

Conclusion: The genes we have identified not only provide candidates for genetic analyses, but will also provide insight into the biological pathways that lead to degeneration of motor neurons in ALS.

C8 INCREASING AUTOPHAGY RESCUES NEURODEGENERATION IN FLIES LACKING ADAR RNA EDITING

PARO S, LI X, MCGURK L, O'CONNELL M, KEEGAN L

MRC-Human Genetics Unit, Edinburgh, United Kingdom

E-mail address for correspondence: simona.paro@hgu.mrc.ac.uk

Keywords: RNA editing, autophagy, Drosophila

Background: It has been shown that a reduction in RNA editing at the *GluR2* Q/R site by ADAR2 is observed in motor neurons (MNs) of patients with sporadic ALS. This lack of RNA editing causes glutamate excitotoxicity and subsequently neuronal death. It has been shown that accumulation of altered mitochondria and intracellular aggregates within amyotrophic lateral sclerosis (ALS) MNs were associated with a defect in autophagy. One possibility is that a reduction in autophagy activation in MNs could contribute to ALS pathogenesis. Autophagy is a process involved in maintaining cellular homeostasis. It degrades long-lived proteins and organelles by enclosing them in a double-membrane vesicle (autophagosome) and delivering them to lysosomes for digestion. Increased numbers of autophagic vacuoles (AVs) are seen in a variety of stress and pathological states. The biological role of autophagy and its relationship to cell death in general and to excitotoxic neuron death in particular is still poorly understood.

Objectives: We have studied the effect of loss of ADAR RNA editing in neurons using *Drosophila*. ADAR2 has one homolog gene in *Drosophila melanogaster*. Immediately upon eclosion from the pupa, $Adar^{5G1}$ null flies show locomotion defects and later develop age-dependent neurodegeneration. EM analysis of both brains and retina of mutant flies reveals abnormal intracellular double-layer membrane vacuoles containing cytosolic material. This study aims to elucidate the pathways that cause neurodegeneration in *Adar* null flies using *Drosophila* genetics.

Methods: A genetic screen for dominant suppressors of reduced $Adar^{5G1}$ viability was performed. We crossed virgin females of *y*, $Adar^{5G1}$, *w*/*FM7* with males from a series of DrosDel strains. We calculate $Adar^{5G1}$ male viability relative to *FM7, Bar* in the presence of the hemizygous DroDel deletion that might show suppressive and/or enhancing effects on viability. The mutant flies that had an increase of viability were aged for 30 days; locomotion activity was assayed using a two-minute open-field locomotion test and hematoxylin and eosin staining of the brain sections was used to investigate neurodegeneration.

Results: The screen revealed that decreasing *Tor* expression with either DrosDel deficiencies or specific P-element insertions in *Tor* suppresses *Adar* mutant phenotypes. The same effect was observed by inducing autophagy by overexpression of *Atg* genes using the *Cha-Gal4* driver. Furthermore viral anti-apoptotic protein p35 expression did not rescue the phenotypes. Additionally, TUNEL-positive apoptotic brain cells were not detected in $Adar^{5G1}$ flies.

Conclusions: For the first time we provide *in vivo* evidence that $Adar^{5G1}$ mutant phenotypes are associated with the autophagy. We did not find evidence of neuron death and autophagy appears to be protective in flies. Autophagy may not be the cause of death in ALS motor neurons but may be protective.

C9 MICRORNA DYSREGULATION IN HUMAN SPORADIC ALS

LIOU L[1], KIM JM[2], RAVITS J[2], MÖLLER T[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Benaroya Research Institute at Virginia Mason, Seattle, WA, United States*

E-mail address for correspondence: moeller@uw.edu

Keywords: microRNA, motor neuron biology, laser capture microdissection

Background: MicroRNAs are noncoding single-stranded RNA molecules about 22 nucleotides in length that function in the post-transcriptional regulation of gene expression by binding to mRNAs. Many genes contain predicted microRNA target sites in their 3'-untranslated regions (UTRs), and computational estimates suggest that about one-third of all genes that encode proteins may be regulated by microRNAs. MicroRNAs have been implicated in neurodegenerative diseases, but relatively little is known about the dysregulation of CNS-specific microRNAs. Differential expression of microRNAs has been seen in brain and CSF from Alzheimer's disease patients, and specific microRNAs have been found to be involved in spinocerebellar ataxia and Parkinson's disease. However, little is known about the role of microRNAs in ALS.

Objectives: To investigate if ALS is associated with a dysregulation of microRNAs.

Methods: Motor neurons and motor neuron-depleted anterior horns were collected from lumbar sections by Laser Capture Microdissection from 10 sporadic ALS patients and 10 controls. Total RNA was isolated using the RNAqueous Micro kit (Ambion). The microRNA expression was assessed using the microRNA TaqMan® qPCR Megaplex pools array (AB) with preamplification according to the manufacturer's protocol. Data analysis was performed with DataAssist V2.0 software (AB).

Results: We found that in motor neurons from control patients on average 104 microRNAs were detectable. In contrast in motor neurons from sporadic ALS patients only 86 miRNA were detectable. The expression level of still detectable miRNA was considerably reduced and no miRNA showed increased expression. In contrast, similar analysis on the surrounding anterior horn depleted of motor neurons, showed no statistically significant difference between ALS and control tissue.

Discussion: We have found a novel and seemingly general (all 10 tested sporadic ALS patients) dysregulation in microRNA levels in sporadic ALS. We are currently investigating the potential underlying mechanisms which might lead to a global reduction in microRNAs (eg expression, processing, localization).

Conclusions: Sporadic ALS is associated with a motor neuron-specific loss in miRNA expression. Our finding might provide novel avenues to investigate the motor neuron loss in ALS.

SESSION 2B AUTONOMY AND QUALITY OF LIFE

C10 QUALITY OF LIFE: INDIVIDUAL VALUES, STANDARDIZED ASSESSMENT?

COHEN SR

Lady Davis Research Institute, Jewish General Hospital, Quebec, Canada, McGill University, Quebec, Canada

E-mail address for correspondence: robin.cohen@mcgill.ca

Keywords: quality of life, assessment, carer

Quantitative assessment of quality of life must begin by ensuring that instruments to measure it include all the relevant content and only relevant content. Otherwise one can measure something with precision, but it will not be quality of life that is measured. However, quality of life can be a nebulous concept. Who decides what is relevant? Is it even possible to have a standardized measure given differences among individuals in terms of what is important to their quality of life? How can there be a good quality of life if one has motor neuron disease, or is caring for someone with it?

Much of the current literature regarding quality of life for people with motor neuron disease does not distinguish between quality of life and health status, and the field may therefore be heading down the wrong path. The following will be explored in this talk. 1) The concept of quality of life, and its relationship to other important outcomes such as health status. 2) Critical questions that need to be answered before embarking on quality of life studies. 3) Different purposes for measuring quality of life and the implications of this for quality of life assessment. 4) The state of quality of life assessment for people with motor neuron disease and for their carers. The talk will conclude with a proposal for the way forward.

C11 THE OREGON DEATH WITH DIGNITY ACT: WHY DO PATIENTS REQUEST PHYSICIAN-ASSISTED DEATH?

GANZINI L

Oregon Health and Science University (OHSU), Portland, OR, United States

E-mail address for correspondence: ganzini@ohsu.edu

Keywords: euthanasia, end of life care, terminal illness

The Oregon Death with Dignity Act, which legalized physician-assisted death (PAD) for terminally-ill patients, was enacted 13 years ago. This law allows a competent, requesting patient, with an estimated life expectancy of less than six months, to receive a prescription for a lethal medication from a physician for the purposes of self-administration. Support for the law varies among Oregon health professionals ranging from 40% of hospice chaplains and 51% of physicians, to 78% of psychologists. Only one third of Oregon physicians are willing to prescribe under the law. On the other hand, nine in ten Oregon hospice nurses who oppose the law would not actively oppose a client's choice for PAD.

Since enactment of the law, 460 patients have died by lethal medication, approximately 2/1000 Oregon deaths. Patients who die by PAD are more likely to have a college education, and a scant 2% lack medical insurance. Although only 35 ALS patients have died by PAD, ALS is the disease associated with the highest likelihood of PAD; ALS patients are over 30 times more likely to use PAD compared to other Oregon decedents. Over half of ALS patients in Oregon indicate they might consider legalized PAD, especially men, those who have high scores on measures of hopelessness, and those who are not religious. Studies of physicians and hospice workers who have cared for requesting patients; family members; and patients themselves, all point to the importance of staying in control, not being dependent on others, maintaining self-sufficiency and not burdening family as reasons patients pursue PAD. Among ALS patients negative views on the future predict persistent interest in PAD over time. Most requesting patients do not have major depressive disorder, however, a small number of patients with depression, who have not been evaluated by a mental health professional, access PAD. Over 80% of patients who have died by PAD in Oregon are hospice enrolled. Hospice referral and provision of non-judgemental support are the most effective interventions resulting in patients finding alternatives to PAD.

Among those whose family member requested PAD, whether or not the patient accessed a lethal prescription had no influence on subsequent depression, grief, or mental health services use; however, family members of Oregonians who received a lethal prescription were more likely to believe that their loved one's choices were honored and less likely to have regrets about how the loved one died. Family members of Oregonians who requested PAD felt more prepared and accepting of the death than comparison family members.

C12 DO RELIGIOSITY AND SPIRITUALITY CORRESPOND WITH ALS PATIENTS' VIEWS ON END-OF-LIFE ISSUES? AN EXPLORATIVE STUDY WITH ALS PATIENTS AND THEIR PRIMARY CAREGIVERS

STUTZKI R[1], WEBER M[2], REITER-THEIL S[1]

[1]*Department of Medical and Health Ethics, Medical Faculty, University of Basel, Basel, Switzerland,* [2]*Neuromuscular Diseases Unit/ALS Clinic of the Cantonal Hospital, St. Gallen, Switzerland*

E-mail address for correspondence: ralf.stutzki@unibas.ch

Keywords: religiosity, spirituality, suicide

Background: In the secular society of Switzerland self-determination, especially regarding end-of-life decisions, is highly esteemed. As suicide and also assistance to suicide are not legally punishable acts (unless in cases of 'selfish motivation'), health care professionals as well as family members or friends of severely ill patients are facing ethical dilemmas, when the patient asks them to help terminating his/her life. This has been observed in groups of patients with malignancies and progressive diseases, including those suffering from ALS (Amyotrophic Lateral Sclerosis).

Objectives: To determine the correlation of personal faith, religious denomination and spirituality with ALS patients' views on end-of-life issues.

Methods: Explorative interview study with 30 patients and their primary caregivers; semi-quantitative questionnaire and qualitative interview study (2008-2010).

Measures: Demographics; Questions on end-of-life decisions; Hospital Anxiety and Depression Scale (HADS); Idler Index of Religiosity (IIR); Schedule for Meaning in Life Evaluation (SMiLE); The Neurobehavioural Rating Scale NRS on quality of life, feeling lonely, being a burden to others; semi-structured, tape-recorded interviews.

Results: Thirty patients and their caregivers were interviewed. The median age of the patients (10 female and 20 male) was 59 years. 15 patients were Roman-Catholic, 10 Protestant and 5 non-denominational. Median age of the care givers was 56 years (10 male, 20 female). 14 caregivers were Roman-Catholic, 7 Protestant and 9 non-denominational. Significant differences between patients and caregivers were found in questions concerning quality of life (P=0.004), loneliness (P=0.005), religious self-assessment (P=0.001) and life-prolonging measures (PEG: P= 0.007). Thirty seven per cent of the patients had already either thought about or discussed the option of ending his or her life with the help of a relative, close friends, pastor or medical doctor. At the same time, none of the interviewees showed any sign or interest to commit suicide or actively asked for assistance to terminate their life.

Discussion: The results suggest that patients affiliated to a religious denomination are less likely to consider suicide and also assistance to suicide as an option of action in comparison with patients who are not practising any faith.

Conclusion: Religious confessional faith does have an impact on a patient's view towards end-of-life issues. There is a significant difference, however, between Roman-Catholic and Protestant patients' views.

C13 THE DIAGNOSTIC JOURNEY FROM SYMPTOM ONSET: EXPERIENCES OF PEOPLE WITH MND AND FAMILY CARERS

O'BRIEN M[1], WHITEHEAD B[1,2], MITCHELL D[2], JACK B[1]

[1]*Edge Hill University, Ormskirk, United Kingdom,* [2]*Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom*

E-mail address for correspondence: obrienm@edgehill.ac.uk

Keywords: diagnostic journey, personal experiences, qualitative research

Background: Studies addressing the diagnosis in MND polarise upon diagnostic delays (1) or communication of the diagnosis (2). There has been little investigation of the journey from symptom onset through to diagnosis as a means of capturing and exploring the full diagnostic experience for patient and family.

Objectives: To use qualitative methodology to explore the personal experiences of patients and family carers to capture the diagnostic journey from initial symptom development through to delivery of the diagnosis. Additionally we sought to identify issues which may impact positively or negatively on these experiences.

Methods: Narrative interview data was collected from a purposively selected sample of people with MND (n=24), current (n=18) and former family carers (n=10). Thematic analysis was aided by NVIVO 7.

Results: A number of themes were apparent. Delays in arriving at a diagnosis arose from a failure to recognise the significance of intermittent and insidious symptoms which were frequently dismissed by patients, carers and health professionals. A lack of urgency within primary care resulted in protracted periods of time between initial GP consultation and onward referral for specialist investigations. Within secondary care there were extended waiting times for investigations. There was consensus amongst participants that these interrelated factors impacted on patients' and carers' well-being. Participants reported a mixture of experiences surrounding the communication of the diagnosis; for some the process was handled sensitively with appropriate explanation and empathy. However, many described interaction at this time as blunt and unsatisfactory revealing poor communication skills and a lack of consideration for the impact of the diagnosis on the patient and their family. Although the diagnosis was traumatic, it came as a relief for some to have their condition named so they could finally understand the cause of their symptoms. Comparisons were made with the assistance available for people with cancer; the impression being that support was routinely provided at the point of diagnosis in cancer, but not so in MND. Previous knowledge of MND did have an impact on the reaction to the diagnosis for a number of participants.

Conclusions: This study has provided insight into the experiences of people affected by MND during the time from symptom onset through to diagnosis. It highlights areas where delays have occurred and reveals the impact on patients and carers of these delays. The need for better and more sensitive healthcare interaction with patients and carers at this traumatic time is apparent.

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C14 COMMUNICATION STRATEGIES THAT ALS PATIENTS USE AT END OF LIFE: RESULTS OF A THREE YEAR SURVEY

BROWNLEE A[1,2], BRUENING L[3]

[1]*ALS Association, National Office, Calabasas Hills, CA, United States,* [2]*ALS Center, University of Pennsylvania Health System, Philadelphia, PA, United States,* [3]*ALS Association, Northern Ohio Chapter, Cleveland, OH, United States*

E-mail address for correspondence: abrownlee@alsa-national.org

Keywords: communication, end of life, AAC

Background: Many people with ALS are not able to verbally communicate at end of life and rely on many different forms of communication systems to address medical and social needs, ask questions, and convey fears and concerns about the dying process. Many allied healthcare professionals have expectations that patients will utilize an electronic communication device until death. The results of this three year survey find that people with ALS are not using electronic communication systems at end of life but in fact are using multiple modalities of communication, the primary system being low technology like eye blinks, gestures, and letter boards. The speaker will present the results of this survey and recommendations to the field to address the communication issues of people with ALS at end of life.

Objectives: 1) Determine how many ALS families were aware of speech/communication issues related to ALS; 2) List specific communication related topics people with ALS are

communicating at end of life; 3) Identify what communication strategies people with ALS are using at end of life; 4) Identify the number of people that were enrolled in hospice.

Methods: Surveys were sent to caregivers of ALS patients in various US States for a three year period from 2007-2010. Caregivers we asked specific questions as to how their loved one was communicating 3 months prior to death, 1 month prior to death, and the last few weeks and days of life. Surveys were sent via regular US mail.

Results: Almost 75% of those surveyed had signed on to hospice services; most people with ALS were communicating via speaking 2-6 months prior to death and 4 weeks prior to death. Days before death, most people with ALS were using gestures, eye blinks, letterboards, and electronic communication devices. In the last 2-6 months, less than 20% of people that had electronic devices used them. In the initial survey, sent to caregivers from 2007-2009, there were a total of 93 caregivers who reported their loved one had an electronic device in the home. 48 caregivers reported that their loved one had a device and chose not to use it – over 50%. Another 19 reported that their loved one sometimes chose not to use it for a total of 72% that had a device and chose not to use it. Most people with ALS used their electronic devices for less than one month.

Conclusions: More stress needs to be placed on low technology options and/or multi-modalities of communication systems. Caregivers and allied healthcare professionals need to be trained in various low technology options since it seems families use multiple systems for communication when people with ALS are near the end of life.

C15 AN ADVANCE-CARE PLANNING DECISION AID FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

BROTHERS A, GREEN M J, LEVI B, FARACE E, SIMMONS Z

Penn State College of Medicine/Hershey Medical Center, Hershey, PA, United States

E-mail address for correspondence: abrothers@hmc.psu.edu

Keywords: advance care planning, computer-based decision aid, end-of-life

Background: Patients with ALS inevitably face decisions about what medical treatments they would and would not want in the future. Common methods of advance care planning have not been particularly effective at facilitating such decision-making. We anticipated that a computer-based decision aid would help ALS patients with the process of advance care planning.

Objectives: To determine whether: 1) a computer-based decision aid improves knowledge and decision-making among patients with ALS; and 2) the intervention increases the multidisciplinary ALS Team's awareness of their patients' healthcare wishes.

Methods: Patients meeting El Escorial criteria for ALS were enrolled in the study. Participants completed pre- and post- intervention questionnaires, using a computer-based decision aid to generate advance directives. Members of the ALS Clinic team (neurologist, nurse, counselor, chaplain, social worker, physical and occupational therapists, speech/language pathologist, dietitian) were surveyed about what end-of-life treatment decisions they thought they would make based on 3 hypothetical vignettes, and a concordance score was calculated to determine the extent to which patients agreed with the ALS team's decisions. Paired-sample t-tests were conducted for the pre-and post-intervention outcome measures and concordance ratings. Descriptive statistics are reported for participants' decisional conflict and satisfaction scores.

Results: There were 18 participants (12 male). 88.9% were married, 94.4% had at least a high-school education, and 94.4% lived in their own home. Participants' knowledge of advance care planning significantly improved after using the decision aid (51.4% correct responses pre-intervention compared to 67.9% post-intervention; $P < 0.001$). Mean (M) Self Determination scores (feeling of control about what medical treatments one will receive in the future) did not change pre- and post-intervention ($M=35$, maximum score=40). Anxiety also remained unchanged ($P = 0.64$). Post-intervention Decisional Conflict (maximum score =80) was relatively low ($M=29$, $SD = 8.3$), indicating that participants felt clear about their decisions for end-of-life care. Satisfaction with the computer program (maximum score=60) was high (mean=52.2, SD = 5.8); similarly, participants were satisfied with their decisions ($M=9.9$, $SD = 3.1$; where 6 = highest satisfaction and 30 = lowest satisfaction). Decisions for end-of-life care made by the patient and by the ALS team (n=11) showed significantly higher post-intervention concordance (maximum score = 100%; pre-intervention $M=61.6$, $SD=31.4$ compared to post-intervention $M=88.4$, $SD=13.5$, $P = 0.017$) suggesting that the ALS team's understanding of patients' wishes significantly improved after the intervention.

Discussion and conclusions: Use of the computer-based decision aid effectively increased participants' knowledge about advance care planning without an increase in anxiety levels. It was favourably received by ALS patients, who indicated high levels of satisfaction with the computer program, and with their decisions. Finally, the intervention was effective in improving the ALS Clinic team's understanding of patients' wishes.

SESSION 3A LESSONS FROM OTHER DISEASES

C16 RNA PROBLEMS AND SOLUTIONS: LESSONS FROM MYOTONIC DYSTROPHY

THORNTON C

University of Rochester, Rochester, NY, United States

E-mail address for correspondence: Charles_Thornton@urmc.rochester.edu

No abstract available.

C17 THE ROLE OF RNA SPLICING IN SPINAL MUSCULAR ATROPHY

PELLIZZONI L

Department of Pathology and Cell Biology, Columbia University, New York, NY, United States, Center for Motor Neuron Biology and Disease, Columbia University, New York, NY, United States

E-mail address for correspondence: lp2284@columbia.edu

Keywords: spinal muscular atrophy, survival motor neuron, RNA splicing

Spinal muscular atrophy (SMA) is an inherited motor neuron disease and the leading genetic cause of infant mortality. SMA is caused by reduced levels of the survival motor neuron (SMN) protein, a core component of a macromolecular complex that is required for the efficient and specific assembly of small nuclear ribonucleoproteins (snRNPs)-the essential constituents of the pre-mRNA splicing machinery. Although SMN is ubiquitously expressed and snRNP biogenesis is required in all cells, we found that SMN activity in snRNP assembly is regulated in a tissue-specific and time-dependent manner, suggesting a differential requirement for this function in distinct cell types and during development. Analysis of the functional consequences of SMN deficiency in mouse models of SMA revealed that snRNP assembly is defective and the extent of its reduction correlates with disease severity. Surprisingly, rather then causing a uniform decrease in the levels of all snRNPs, SMN deficiency causes tissue-specific changes in the snRNP profile of SMA tissues by unevenly altering the normal proportion of endogenous snRNPs. Furthermore, the levels of a subset of snRNPs that are responsible for the splicing of a rare class of introns (U12-type) representing less than 1% of all eukaryotic introns are particularly affected in SMA tissues. Based on this observation as well as the finding that restoration of a normal snRNP profile coincides with phenotypic correction in animal models of SMA, we investigated a possible link between SMN-dependent defects in the U12 splicing pathway and SMA etiology. Using cellular and animal models we found that SMN deficiency affects splicing of U12 introns in a significant proportion of genes with this type of introns. We took advantage of the very small number of U12 intron-containing genes to carry out a genome-wide functional analysis in *Drosophila* mutants of SMN. In this model of SMA, SMN deficiency causes synaptic dysfunction at the neuromuscular junction (NMJ), decreased muscle size and impaired locomotor activity. We have found that expression of approximately 40% of U12 intron-containing genes is decreased in *Drosophila* SMN mutants and studied the contribution to the neuromuscular phenotype of each of these genes. Using RNAi inhibition and overexpression in specific neuronal types, we have identified a novel transmembrane protein whose restored expression in *Drosophila* mutants of SMN fully rescues neurotransmitter release at the NMJ and significantly improves muscle size and locomotion. These findings reveal that SMN deficiency can elicit neuronal dysfunction at the NMJ by affecting splicing of genes critical for synaptic transmission and support the conclusion that SMA is an RNA splicing disease.

C18 MUTANT SMALL HEAT-SHOCK PROTEIN 27 (HSPB1) MICE RECAPITULATE AXONAL CHARCOT-MARIE-TOOTH DISEASE AND DISTAL HEREDITARY MOTOR NEUROPATHY PHENOTYPES

D'YDEWALLE C[1], KRISHNAN J[1], CHIHEB D[1], VAN DAMME P[1,2], IROBI J[3,4], VAN DEN BERGHE P[5], TIMMERMAN V[3,4], ROBBERECHT W[1,2], VAN DEN BOSCH L[1]

[1]*VIB and University of Leuven, Leuven, Belgium,* [2]*University Hospital Leuven, Leuven, Belgium,* [3]*VIB & University of Antwerp, Antwerp, Belgium,* [4]*Institute Born Bunge, Antwerp, Belgium,* [5]*University Leuven, Leuven, Belgium*

E-mail address for correspondence: constantin.dydewalle@vib-kuleuven.be

Keywords: Charcot-Marie-Tooth disease, peripheral neuropathy, mouse models

Background: Charcot-Marie-Tooth (CMT) disease is the most common inherited disorder of the peripheral nervous system. CMT patients show progressive distal muscle weakening and atrophy, foot deformities and sensory loss leading to steppage gait. CMT is classified into three groups: demyelinating (CMT1), axonal loss (CMT2) and mixed forms. When exclusively lower motor neurons are affected, CMT2 is referred to as distal hereditary motor neuropathy (distal HMN). Mutations in the gene encoding the small 27 kDa heat-shock protein (HSPB1) have been identified as the cause of CMT and/or distal HMN.

Objectives: To elucidate the pathological mechanism underlying mutant HSPB1-induced CMT2 and/or distal HMN *in vivo*, we aimed to develop and characterize transgenic mice expressing wild type (wt) or mutant HSPB1 selectively in neurons.

Methods: We created transgenic mice expressing wt and two different mutant (S135F and P182L) HSPB1 isoforms driven by a Thy1.2 expression cassette. Several phenotypical tests (including rotarod, grip strength, hotplate, gait analysis and nerve conduction studies) were used to characterize these transgenic mice. Real-time imaging of mitochondria was performed on DRG neurons isolated from symptomatic mice to assess axonal transport.

Results: S135F- and P182L-HSPB1 lines demonstrated limb-clasping behavior from the age of 6 months on. Both mutant HSPB1 lines showed reduced rotarod performance aggravating in function of age. Muscle force was severely affected in hind limbs from 6 months on, while forepaw muscle force decreased only later in disease in both mutant HSPB1 lines. Mutant HSPB1 mice demonstrated steppage gait. In contrast to P182L-HSPB1 mice, S135F-HSPB1 mice showed sensory loss as assessed by the hotplate test. Nerve conduction studies revealed signs of motor axonopathy in

P182L-HSPB1 mice, and mixed sensorimotor axonopathy in S135F-HSPB1 mice. Histochemical analysis of muscle, neuromuscular junctions and peripheral nerves showed signs of peripheral neuropathy in both mutant HSPB1 lines. Mitochondrial transport was severely affected in DRG neurons isolated from symptomatic S135F-HSPB1 mice, but not from P182L- and wt-HSPB1 mice.

Discussion: The selective expression of mutant HSPB1 in neurons gives rise to CMT2 or distal HMN phenotypes in mice. Thus, mutant HSPB1 cell-autonomously leads to a peripheral neuropathy. S135F-HSPB1 mice display all key features of a mixed sensorimotor polyneuropathy while P182L-HSPB1 mice only demonstrate signs of a motor neuropathy. These findings are in line with the clinical symptoms in CMT2 and distal HMN patients, respectively. Disturbances of mitochondrial transport along peripheral axons might underlie the pathological mechanism of CMT2 and distal HMN.

Conclusion: This work describes new mouse models for CMT2 and distal HMN. These models accurately recapitulate all key symptoms of both human conditions, depending on the mutation in HSPB1. Moreover, these models provide powerful tools to elucidate the pathological mechanism underlying CMT2 and distal HMN, paving the way to develop therapeutical strategies.

SESSION 3B CARE EDUCATION AND PRACTICE

C19 PALLIATIVE CARE IN TERMINAL ILLNESS - THE IMPACT ON HEALTHCARE PROFESSIONALS

JACKSON V[1,2]

[1]*Massachusetts General Hospital Department of Medicine, Palliative Care Unit, Boston, MA, United States,* [2]*Harvard Medical School, Boston, MA, United States*

E-mail address for correspondence: vjackson@partners.org

Keywords: end of life care, physician/patient communication, palliative care education

Caring for patients who are suffering and will eventually die is a particular challenge. Clinicians need to develop ways of coping with this stress that allows a continued empathic, therapeutic connection with the patient and family while promoting a sustainable model of clinical care. It is not uncommon for issues of conflict, grief, appropriate boundaries, and helplessness to complicate these relationships. In this session, the challenges in caring for these patients and methods for addressing this stress will be reviewed.

C20 DEVELOPMENT, IMPLEMENTATION AND TESTING OF AN EDUCATIONAL PROGRAM TO GUIDE PALLIATIVE CARE FOR PEOPLE WITH MOTOR NEURONE DISEASE

MCCONIGLEY R[1], AOUN S[1], KRISTJANSON L[1], CURROW D[2], O'CONNOR M[3], COLYER S[4], EDIS R[5], HARRIS R[6], ALLCROFT P[7], YATES P[8]

[1]*Curtin University of Technology, Perth, Western Australia, Australia,* [2]*Flinders University, South Australia, Australia,* [3]*Monash University, Victoria, Australia,* [4]*MND Association of Western Australia, Western Australia, Australia,* [5]*Royal Perth Hospital, Western Australia, Australia,* [6]*Motor Neurone Disease Association of Victoria, Victoria, Australia,* [7]*Southern Adelaide Palliative Care Services, South Australia, Australia,* [8]*Queensland University of Technology, Queensland, Australia*

E-mail address for correspondence: R.McConigley@curtin.edu.au

Keywords: palliative care, education, evaluation

Background: The relative rarity of MND means that many palliative care professionals will not have sufficient knowledge of MND to provide a high standard of care to patients and families. A large Australian study of the needs of people with four neurodegenerative disorders found that this group of people and their carers had many unmet care needs. People with MND reported the lowest quality of life of the groups studied, as did their carers. Therefore, it is essential that education be provided to palliative care professionals about MND to ensure that care is appropriate.

Objectives: This project aimed to improve the quality of care for people with MND and their families through the development, testing and implementation of an educational program for service providers focused on the palliative care needs of this population.

Methods: This project used a three-phased approach to develop and implement an educational program about providing palliative care for people with MND. Phase 1 involved an extensive consumer consultation process, including conducting interviews and focus groups with patients, carers and health professionals to determine education needs. The second phase involved developing and implementing an educational program for people working in the palliative care area. The third phase involved testing the efficacy of the education program in improving the care of people with MND and their families.

Results: The results of the phase 1 consultation showed that the education program needed to provide information about what MND is and how to effectively manage practical problems experienced by patients and families using an experiential method of teaching. The resulting education package consisted of six one-hour modules that could be presented as stand-alone sessions or as part of a workshop or education series. Six one-day workshops were held to test the education materials developed and assess the effectiveness of the sessions at changing the knowledge and attitudes of participants. Results of the testing showed that following attendance at the workshops participants had increased knowledge of MND and were more likely to have positive attitudes to providing care for people with MND.

Discussion: The results of this study show that palliative care professionals are interested in learning more about MND and that a targeted education program can improve their knowledge and attitudes to providing care to this under-served group. An implementation plan has been developed to ensure widespread uptake of the educational program across Australia.

Conclusions: People living with MND and their families will benefit from increased access to palliative care services that are able to provide specialist care when required. A targeted education program for palliative care professionals will assist in improving the palliative care provided.

C21 PAIN IN ALS: FREQUENCY AND CHARACTERISTICS IN A POPULATION BASED SERIES

CALVO A, CANOSA A, GALLO S, MOGLIA C, BERSANO E, BALMA M, ILARDI A, CAMMAROSANO S, PAPURELLO D, CHIÒ A

Department of Neuroscience, University of Torino, Torino, Italy

E-mail address for correspondence: achio@usa.net

Keywords: pain, population-based, case control

Background: Pain is considered rare in early stages of amyotrophic lateral sclerosis (ALS), but it is more frequently reported during advanced stages. However, there are no studies devoted to the assessment of frequency and characteristics of pain in ALS, compared to a control population.

Aim: The aim of this survey was to determine the characteristics of pain in a consecutive series of ALS patients, comparing it to a control population.

Methods: A total of 120 ALS incident patients from the Piemonte Register for ALS (PARALS) were interviewed. Pain was evaluated with the Brief Pain Inventory (BPI) questionnaire. Patients' physical status was evaluated with ALSFRS-R. The control population included people matched by age

(±3 years) and gender, randomly selected from the patients' general practitioners lists.

Results: Of the 120 ALS patients, 68 were men and 52 women. Their mean age at the time of the interview was 62.2 years, their mean disease duration was 44.4 months (median, 34 months), and their mean ALSFRS-R score was 30.0 (range 0-45). Controls were similar to patients for the main demographic characteristics. Pain was reported by 64 patients (53.3%) and 44 controls (36.7%) (P = 0.009). Maximum and mean pain intensity were similar in patients and controls. Forty-seven patients (73.4%) and 19 controls (43.2%) were treated for pain at the time of the interview (P = 0.0001). The efficacy of therapy for pain received similar rates in patients (63.3%) and controls (66.7%) (P = 0.88). The probability of being treated among patients was not related to the rating of severity pain, but was significantly higher in patients reporting negative effects of pain on social relationship and enjoyment of life. Among patients, the presence of pain was not related to age, gender, but significantly increased with the worsening of disability (ALSFRS-R score) (P = 0.04) and disease duration (P = 0.03). Patients reported that pain caused an interference with their mood and enjoyment of life and to a lesser extent with their social relationships and sleep.

Comments: In our series, pain was more frequent in ALS patients than in age and gender matched controls, although its intensity was similar. The presence was related to patients' disability. More patients than controls received a treatment for their pain, but the control of pain was deemed not completely satisfactory both in patients and in controls.

C22 PATIENT CARE COORDINATION AT CAROLINAS NEUROMUSCULAR/ALS – MDA CENTER: NURSING MANAGEMENT OF E-PATIENTS AND T-PATIENTS

WILLIAMS NM[1], BLACK KJ[1], NICHOLS MS[1], BRAVVER E[1,2], BROOKS BR[1,2]

[1]*Carolinas Medical Center, Department of Neurology Neuromuscular/ALS-MDA Center, Charlotte, NC, United States,* [2]*University of North Carolina, Chapel Hill School of Medicine, Chapel Hill, NC, United States*

E-mail address for correspondence: nicole.m.williams@carolinas healthcare.org

Keywords: tele-nursing, telephone advice, internet web patient portal

Background: Patient care coordination requires a communication infrastructure to provide contact between patients and caregivers with nurses, allied health personnel, physicians and others. Day to day contacts cover a number of patient care, caregiver, care provider needs but little information exists in the ALS literature concerning communication techniques and topics covered in this communications infrastructure.

Objective: To identify baseline communication interactions in a large ALS clinic situated in the third largest public healthcare system in the United States, Carolinas Healthcare System.

Methods: Email (E-patients)and telephone (T-patients) contacts are monitored for one week each quarter and contacts are categorized by subject and interaction type (1). Summary data is analyzed with descriptive statistics.

Results: Total weekly contacts (88.0±7.4 /wk (standard deviation)) by Email (2.3%) or telephone (97.7%) consisted of patient-nurse (71.7±7.5 /wk (80.7%)), nurse-nurse (16.0±1.5 /wk(18.8%)) and drug company representative-nurse (1.0±0.4 /wk(0.5%)) interactions. The bulk (50.0%) of weekly contacts concerned appointments for MD, allied health or test follow ups (44.0±5.6 /wk) while the second most common concern (17.0%) was medications (15.0±2.1 /wk) followed by equipment needs (9.1%) referred to physical, occupational and augmentative communication therapy (8.0±1.1 /wk) and requests (6.8%) for medical records (6.0±2.1 /wk). Changes in patient symptoms (6.0+1.3 /wk(6.8%)), disability formspatient bills (5.0±0.7 /wk(5.7%)), patient laboratory results (4.0±0.4 /wk(4.5%)), home care issues (3.0±0.9 /wk(3.4%)) and patient transport issues (2.0±0.9 /wk(2.3%)) completed.

Conclusions: Nurse-managed average daily patient contacts by Email (0.5±0.2 /d(2.3%)) and telephone (17.6±1.5 /d(97.7%)) require periodic audit to justify allocation of resources. In this ALS Clinic communication contacts for follow-up appointments concerning care issues constituted 50.0% of daily nursing interactions similar to a specialty pediatric clinic (2). This analysis provides benchmark information concerning communication interactions among nurses, patients, other care providers relying primarily upon a telephone-based system prior to development of internet patient portals for more web-based communication with ALS patients.

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C23 A NATIONAL STUDY OF AMYOTROPHIC LATERAL SCLEROSIS MULTIDISCIPLINARY CLINIC UTILIZATION

YOUNG J[1], FELGOISE S[1], STEPHENS HE[2], WALSH S[3], SIMMONS Z[2]

[1]*Philadelphia College of Osteopathic Medicine, Philadelphia, PA, United States,* [2]*Pennylvania State University College of Medicine, Hershey, PA, United States,* [3]*ALS Association, Greater Philadelphia Chapter, Philadelphia, PA, United States*

E-mail address for correspondence: hstephens1@psu.edu

Keywords: multidisciplinary care, quality of life, social problem solving skills

Background: Some literature indicates that patients with ALS who attend multidisciplinary clinics (MDCs) have a higher quality of life (QOL) than those who do not. However, a previous study from our group demonstrated no QOL difference between attendees and nonattendees. Social problem solving (SPS) skills predict QOL in ALS caregivers. Therefore, SPS skills may affect choice of health care delivery or differentiate between those with higher and lower QOL.

Objectives: 1) To investigate differences between persons with ALS who access MDCs and those who do not with regard to: QOL, use of adjuvant therapies and support services, functionality, and SPS skills; 2) To obtain qualitative information to understand reasons why people choose to attend or not attend MDC.

Methods: Participants completed a web-based survey in response to announcements posted on Patientslikeme.com and the ALS Association website. The survey included the ALS Specific Quality of Life Questionnaire-Revised (ALSSQOL-R), the ALS Functional Rating Scale Revised (ALSFRS-R), the Social Problem Solving Inventory Revised (SPSI-R), and questions regarding MDC utilization. Multiple analysis of variance (MANOVA) was used to test for differences between attendees and nonattendees on the ALSSQOL-R and SPSI-R subscales and total scores. Univariate analysis was used to identify differences when the MANOVAs revealed significant differences. T-tests, analysis of variance, and descriptive statistics were used to test differences between the samples on personal characteristics, and to describe these characteristics.

Results: 403 ALS patients completed the survey –314 attendees and 89 nonattendees. Attendees had higher scores than non-attendees for average total ALSSQOL-R (mean 6.35 vs. 5.75, P=0.031). and for the subscales of Intimacy (P = 0.021), Physical Symptoms (P = 0.008), and Bulbar Function (P = 0.021). Both groups' mean scores on SPSI-R and subscales were within the average range, and did not differ from one another (P = 0.147). Reasons for attending MDCs included: 1) Expertise of the specialist, 2) information and knowledge obtained, 3) convenience, travel and time, 4) support, and 5) research and clinical trials. Attendees reported more use of medications for depression, saliva, cramping, spasticity, pain and sleep. They also were more likely to have received services from other health professionals, complementary/alternative medicine, and in-home care.

Discussion: Individuals attending a MDC generally have a better QOL than those who do not. They can identify multiple reasons for attendance, and receive more treatments and services. Problem solving skills do not differ between attendees and non-attendees. It is possible that those who attend MDC are in need of more services and support than non-attendees to maintain QOL, or that those who attend a MDC perceive their QOL as better because they have more support.

SESSION 4A EMERGING DISEASE MODELS

C24 TDP-43 MUTANT TRANSGENIC MICE DEVELOP BIOCHEMICAL AND PATHOLOGICAL FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL LOBAR DEMENTIA

SWARUP V, PHANEUF D, BAREIL C, JULIEN J-P

Centre de Recherche du CHUQ, Department of Neuroscience and Psychiatry, University Laval, Quebec, QC, Canada

E-mail address for correspondence: vivek.swarup@crchul.ulaval.ca

Keywords: TDP-43, mouse models, mutations

Objectives: Neuronal cytoplasmic and intranuclear aggregates of RNA-binding protein TDP-43 are a hallmark feature of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). ALS and FTLD show a considerable clinical and pathological overlap and occur as both familial and sporadic forms. Mutations in TDP-43 are known to cause both the familial and the sporadic form of ALS in about 3% of patients. The goal of this study is to overexpress two such TDP-43 mutations – A315T and G348C and the wild-type human TDP-43 protein in transgenic mice, thereby modeling the human disease.

Materials and methods: TARDBP (NM_007375) was amplified by PCR from a human BAC clone (clone RPCI-11, clone number: 829B14) along with the endogenous promoter (~5kB). Site-directed mutagenesis using the primer-based approach was used for the generation of mutants (TDP-43^{A315T} and TDP-43^{G348C}). The full-length TARDBP (wild-type, G348C and A315T mutants) were linearized by Swa-1 restriction enzyme and microinjected in mice embryos (having a background of C3H X C57BL/6). Later, they were transplanted in pseudo-pregnant mothers (having ICR CD1 background). Founders were bred with Ntg C57BL/6 mice to establish stable transgenic lines. Transgene expression was analyzed in brain and spinal cord by real-time PCR and in brain, spinal cord, muscle, liver by Western blot using monoclonal human TDP-43 antibody (Clone E2-D3, Abnova). Immunohistochemistry and immunofluorescence on spinal cord and brain sections of TDP-43 transgenic mice were done using monoclonal TDP-43 antibody, ubiquitin (Chemicon), Iba-1 (Wako), GFAP (Chemicon).

Results: Neurons in the affected spinal cord and brain regions showed accumulation of TDP-43 nuclear and cytoplasmic aggregates that were both ubiquitinated and phosphorylated as observed in ALS/FTLD patients. Moreover, the characteristic ~25-kDa C-terminal fragments (CTFs) were also recovered from nuclear fractions and correlated with disease development and progression in TDP-43^{A315T} and TDP-43^{G348C} mutant mice as compared to TDP-43^{wt} mice. TDP-43^{G348C} mice showed increased ubiquitin positive TDP-43 aggregates in the cytoplasm. Also these mice had learning and memory deficits as evaluated by passive avoidance test compared to TDP-43^{wt}. TDP-43^{G348C} and TDP-43^{A315T} transgenic mice also had increased microgliosis and astrogliosis.

Conclusion: Our results suggest that mutation in TDP-43 (A315T and G348C) result in cytoplasmic aggregation of TDP-43 and its ubiquitination and phosphorylation. These mice have increased microgliosis and astrogliosis. These mice also have severe learning and memory deficits suggesting their usefulness in modeling ALS and FTLD-U.

C25 A NEW MOUSE MODEL OF ALS CARRYING A POINT MUTATION IN THE MOUSE SOD1 GENE

JOYCE P[1], FRATTA P[2], PHATAK V[1], MCGOLDRICK P[2], GREENSMITH L[2], FISHER EMC[2], ACEVEDO-AROZENA A[1]

[1]*MRC Mammalian Genetics Unit, Harwell, United Kingdom,* [2]*UCL Institute of Neurology, London, United Kingdom*

E-mail address for correspondence: p.joyce@har.mrc.ac.uk

Keywords: SOD1, mouse models, phenotype

Background: The array of SOD1 transgenic models created to study ALS has been extremely useful in furthering our understanding of the disease. However, a potential drawback of these models is that they express SOD1 at higher levels than in human ALS patients. Thus, some of the phenotypes described in SOD1 transgenic animals could be caused by the high level of expression as opposed to the actual effects of mutant SOD1.

Objective: To create a more physiologically relevant model of ALS and try and overcome the disadvantages that underlie the SOD1 transgenic models.

Methods: We screened the Harwell N-ethyl-N-nitrosourea (ENU) archive for mutations in the Sod1 gene. ENU is a potent mutagen that causes genome-wide point mutations. The MRC Harwell ENU archive consists of 10,000 matching DNA and sperm samples from first generation progeny of ENU mutagenised male mice. Following the identification of ENU-generated mutations, mutant mice can be re-created through *in vitro* fertilisation from stored sperm. We have identified an allelic series of ENU induced mutants carrying mutations in the mouse Sod1 gene: D83G, E109G and R115H. All three mutated residues are mutated in ALS cases, with the D83G mutation carrying the same amino acid change. As these are point mutations, animals express SOD1 mutant protein at endogenous levels. Using an array of behavioural, physiological and histological techniques, we have begun to characterise Sod1 D83G homozygote and heterozygote mutant animals.

Results: Phenotyping experiments are ongoing, however, initial behavioural phenotyping of homozygote Sod1 D83G mice has revealed ALS-like defects such as abnormal gait and decreased grip strength, body weight and startle response. Sod1 D83G homozygote mice generate less muscle force and fatigue less, similar to transgenic mice expressing the SOD1 G93A mutant protein. In addition, Sod1 D83G homozygote mice show a reduced number of motor neurons at 15 weeks. Heterozygote D83G mice appear similar to wildtype mice until approximately 7 months, at which time they develop behavioural and physiological abnormalities. Current ongoing work will establish how the Sod1 D83G homozygote and heterozygote animals deteriorate over time.

Discussion and conclusions: The identification of the Sod1 D83G ENU derived mutation provides the ALS community with a new model of disease. Early observations suggest that these animals display motor abnormalities and motor neuron cell death similar to that seen in ALS. Interestingly, the effect of the Sod1 D83G allele appears to be dosage dependent with homozygote animals displaying defects sooner than heterozygotes. This suggests that, even at physiological expression levels, the amount of mutant protein is critical for disease onset. In addition, wildtype Sod1 may be neuroprotective since

homozygote Sod1 D83G mice, which do not express wildtype SOD1, develop ALS-like phenotypes sooner than heterozygotes. In conclusion, mice carrying the Sod1 D83G mutation could represent the next generation of Sod1-ALS mouse models and will be a powerful tool for analysing early stages of disease.

C26 DEVELOPMENT AND CHARACTERISATION OF A ZEBRAFISH MODEL OF MUTANT SOD1 MEDIATED MOTOR NEURON DISEASE

DA COSTA M, ALLEN C, HIGGINBOTTOM A, RAMESH T, MCDERMOTT C

University of Sheffield, Sheffield, South Yorkshire, United Kingdom

E-mail address for correspondence: m.dacosta@sheffield.ac.uk

Keywords: zebrafish, SOD1, oxidative stress

Rationale and hypothesis: Mutations in the superoxide dismutase gene (SOD1) are known to cause familial Amyotrophic Lateral Sclerosis/Motor Neuron Disease (ALS/MND) in humans. SOD1 is a soluble cytoplasmic and mitochondrial protein, which converts superoxide radicals to oxygen and hydrogen peroxide. In order to test potential new therapies for MND, animal models of the disease are required. Zebrafish are an excellent model for human neurological diseases as they give plentiful transparent embryos, which are easy to manipulate and visualize. Furthermore, the human and the zebrafish SOD1 protein share 76% homology.

Methodology: TILLING (Targeting Induced Local Lesions IN Genomes) was carried out in collaboration with the Sanger Institute. DCF assays to detect oxidative stress were performed on NSC34 cells transfected with zfWT and zfT70I *sod1*. Oxidative stress assays were also carried out on 24hpf dechorionated embryos, using survival as a read out of chronic stress induced by H_2O_2, and DCF fluorescence as a read out of acute stress induced by $CuSO_4$. Neuromuscular junction (NMJ) staining using α-bungarotoxin (post-synaptic) and SV2 (pre-synaptic) was carried out on 11dpf larvae.

Results: Through TILLING, we identified a zebrafish carrying the T70I mutation in the *sod1* gene. The zfT70I mutation occurs in the zinc-binding loop of the SOD1 protein and has been shown to significantly confer susceptibility to oxidative stress in both NSC34 cells ($P < 0.001$) and zebrafish embryos ($P < 0.001$). The zfT70I *sod1* mutation also causes a significant reduction in colocalisation of α-bungarotoxin and SV2 in NMJ staining in homozygous 11dpf larvae compared to WT ($P < 0.001$) and T70I heterozygous ($P < 0.05$) clutchmates. Preliminary data suggest that 14 dpf larvae show a motor phenotype, and further behavioural studies using 14 dpf larvae and adult homozygous T70I *sod1* and WT zebrafish are currently ongoing.

Conclusions: In mammalian *sod1* ALS models, *sod1* mutation confers a susceptibility to oxidative stress and this mechanism has been identified as one of the key pathways in ALS pathogenesis. We have demonstrated that homozygous T70I *sod1* embryos are also more susceptible oxidative stress induced by either hydrogen peroxide or copper sulphate. At 11 dpf, disruption of the neuromuscular junction is observed in T70I *sod1* larvae. This is consistent with the current understanding of ALS pathogenesis in man, where the early pathology is observed in the neuromuscular junction. Our data therefore support the pathogenicity of the T70I *sod1* mutation and that the zebrafish can be used as a model demonstrating key features of ALS pathogenesis. Our zebrafish model is also unique in that the TILLING missense mutation is more akin to MND patients with *sod1* mutations than current transgenic murine models of MND which rely on high levels of over-expression of the h*sod1* transgene and the mutant SOD1 protein.

C27 NOVEL ZEBRAFISH MODELS TO INVESTIGATE ALS DISEASE PATHOGENESIS

SAKOWSKI S, BUSTA A, PHILIP S, ROSENBERG A, DOWLING J, FELDMAN E

University of Michigan, Ann Arbor, MI, United States

E-mail address for correspondence: staceysa@med.umich.edu

Keywords: zebrafish, neuroprotection, SOD1

Background: Amyotrophic lateral sclerosis (ALS) is a lethal multifaceted disease involving a complex interplay among many cell types and the unknown mechanisms of motor neuron (MN) degeneration in ALS make designing specific therapies challenging. In order to develop much needed therapies for this devastating disease, we have generated novel zebrafish models of ALS. Detailed observations of peripheral events occurring in MN axons and at neuromuscular junctions (NMJs) are possible in zebrafish, and they are permeable to small molecules and drugs, making them an ideal system for these studies.

Objectives: The objectives of this study are to generate novel *in vivo* models of ALS by expressing two forms of mutant SOD1 associated with familial ALS in zebrafish. G93A-SOD1, the most commonly studied SOD1 mutation, and A4V-SOD1, the most prevalent mutation in North America which is associated with a highly aggressive disease course, will be used to generate transient and stable transgenic zebrafish models.

Methods: Transient-transgenic zebrafish expressing G93A-SOD1 and A4V-SOD1 are generated by RNA microinjection following timed mating of wildtype AB zebrafish. Effects on early MN axonal development are examined by quantifying axon length following immunohistochemistry. Stable transgenic zebrafish expressing G93A-SOD1 and A4V-SOD1 are generated by Tol2-mediated transgenesis. Swimming ability is examined using the Noldus Larvae Activity Monitoring System to characterize symptom onset, and morphological characterization of NMJ and MN integrity is examined by immunostaining throughout the disease course in stable transgenic zebrafish.

Results: Mutant SOD1 expression results in a dose-dependant decrease in MN axon length in transient-transgenic zebrafish. Co-injection of RNA for insulin-like growth factor-I (IGF-I) upregulates neuroprotective pathways and rescues the phenotype. Stable transgenic zebrafish expressing G93A-SOD1 exhibit a progressive loss of swimming ability, indicative of symptom onset, around 15 weeks of age. Correlation of NMJ integrity and MN loss with symptom onset in stable transgenic zebrafish provide an established model for mechanistic and therapeutic discovery.

Discussion and conclusions: Rescuing the MN phenotype in zebrafish transiently expressing G93A-SOD1 and A4V-SOD1 using IGF-I validate the use of mutant SOD1 for the generation of stable transgenic ALS zebrafish models. Our successful development and characterization of transgenic ALS zebrafish enable a detailed look into the symptomatic and morphologic ALS disease course. Zebrafish can be obtained in large numbers, develop quickly, and are permeable to small molecules and drugs; therefore, transgenic ALS zebrafish provide a valuable vertebrate model to screen much-needed therapies. Knowledge obtained from these studies

using transgenic ALS zebrafish will greatly advance our ability to treat ALS by identifying points for therapeutic intervention throughout the course of MN degeneration, and provides a novel *in vivo* system for therapeutic discovery.

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C28 MUTANT HFE H63D PROTEIN IS ASSOCIATED WITH PROLONGED ER STRESS AND DECREASED NEURONAL VIABILITY IN ALS

LIU Y[1], LEE S[1], NEELY E[1], NANDAR W[1], SIMMONS Z[2], CONNOR J[1]

[1]*Department of Neurosurgery,* [2]*Department of Neurology; Penn State College of Medicine, M.S. Hershey Medical Center, United States*

E-mail address for correspondence: yliu2@hmc.psu.edu

Keywords: HFE, ER stress, neuronal viability

Background: Endoplasmic reticulum (ER) stress appears to be present in patients with amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. Accumulation of mis-folded proteins in the ER provokes the early ER stress response – unfolded protein response (UPR). UPR is a short-term and protective homeostatic mechanism. It initiates both apoptotic and adaptive pathways. It is not clear whether ER stress is largely neuroprotective or whether it directly contributes to the disease process in ALS. The HFE gene encodes for an iron regulating protein, and the H63D variant of this gene is found in increased frequency in patients with ALS and may increase the risk of ALS by four-fold.

Objectives: To determine the ER stress activation pattern associated with HFE H63D mutant protein and its contribution to neuronal viability, as the first step in understanding the mechanisms by which HFE H63D increases the risk for ALS.

Methods: We developed inducible neuronal cell lines expressing HFE wild type (WT) or H63D protein. This system allows us to faithfully capture the transit responses and follow the shift of those responses after the induction. We also expand our *in vitro* findings to a knock-in mouse model carrying the HFE H67D mutation, the mouse equivalent of human H63D.

Results: Our data demonstrated that the presence of HFE H63D mutant protein initially evoked UPR, as revealed by the elevated levels of the major UPR sensors. This response was followed by a persistent ER stress, as the signals of UPR sensors attenuated. At this time point, both Caspase-3 cleavage and activity were up-regulated. This ER stress pattern associated with HFE H63D was also seen in a HFE H67D knock-in mouse model, in which UPR was selectively activated in the lumbar spinal cord at 6-months then declined at 12-months, in association with increased Capase-3 cleavage. Based on the MTS assay, the number of cells in proliferation was decreased in the HFE H63D expressing cells, but no increased cytolysis was detected by measuring LDH activity in the medium. Thus, the data indicated that HFE H63D expressing cells had decreased cell proliferation compared to WT, but did not undergo extensive cell death. Additionally, in spite of increased iron level in cells carrying HFE H63D, it appeared that ER stress was not responsive to the change of cellular iron status.

Discussion and conclusions: Our studies indicate that the HFE H63D mutant protein is associated with prolonged ER stress and reduced cell proliferation, reflecting decreased neuronal viability. We speculate that in the case of HFE H63D, sustained ER stress attenuates the activation of the cytoprotective UPR and thus limits the ability to remove mis-folded proteins, creating an environment which is more conducive to the development of ALS.

C29 DYSREGULATION OF ER STRESS SIGNALING BY ALS-RELATED P56S VAPB MUTANT

KANEKURA K, SUZUKI H, LU S, KANEKURA M, MATSUOKA M, AISO S, URANO F

University of Massachusetts Medical School, Worcester, MA, United States

E-mail address for correspondence: Fumihiko.Urano@umassmed.edu

Keywords: VAPB, ER stress, unfolded protein response

Background: P56S mutation in the VAPB gene causes autosomal-dominant ALS and spinal muscular atrophy. VAPB is a type II transmembrane protein localized to endoplasmic reticulum (ER) whose function remains elusive. We previously reported that VAPB is involved in the unfolded protein response (UPR), an adaptive response to ER stress, and ALS-related P56S VAPB acts as a dominant-negative mutant, resulting in motor neuron vulnerability to ER stress.

Objectives: 1) To investigate the precise mechanisms by which VAPB regulates the UPR. 2) To investigate how P56S VAPB mutant causes motor neuron vulnerability to ER stress.

Methods: To investigate how VAPB regulates the UPR, we developed a lentivirus-based inducible system of wild-type and P56S mutant VAPB in NSC34 cells. We monitored activation levels of the UPR by measuring expression of ER stress markers and activation of the UPR luciferase reporter in these cells. To identify genes and pathways required for VAPB to regulate the UPR, we established a genome-wide shRNA screening system.

Results: We found that VAPB plays a role in activating the IRE1-XBP-1 pathway and suppressing the PERK-ATF4 pathway of the UPR. In contrast, P56S VAPB mutant strongly activates the PERK-ATF4 pathway and causes upregulation of CHOP, a pro-apoptotic component of the UPR. Furthermore, we found that FFAT-motif containing oxysterol binding family proteins are required for VAPB to activate the IRE1-XBP-1 pathway. We are currently conducting a genome-wide shRNA screen to identify genes required for VAPB to regulate the UPR.

Discussion and conclusions: VAPB acts as an activator for the IRE1-XBP-1 pathway and a suppressor for the PERK-ATF4 pathway of the UPR. The ALS-related P56S VAPB mutant acts as a dominant-negative mutant, leading to the activation of the pro-apoptotic PERK-ATF4-CHOP pathway. Our results indicate that dysregulation of the UPR caused by P56S mutant VAPB plays a role in the pathogenesis of ALS.

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SESSION 4B SURROGATE MARKERS

C30 VALIDATION OF A NEW DEVICE TO MEASURE DISEASE PROGRESSION IN ALS

ANDRES P[1], SKERRY L[1], CUDKOWICZ M[1,2]

[1]*Massachusetts General Hospital, Boston, MA, United States,* [2]*Harvard Medical School, Cambridge, MA, United States*

E-mail address for correspondence: pandres1@partners.org

Keywords: outcomes measures, quantitative strength measures, disease progression

Background: Currently used outcome measures require large sample sizes and may be unable to detect small, but clinically important, treatment effects. Maximal voluntary isometric (MVIC) strength testing accurately reflects ALS disease progression. However, MVIC, using a strain gauge, is inconvenient and expensive. Hand held dynamometry is convenient but limited by the evaluator's strength.
To address the need for a convenient, accurate method to test MVIC, we developed a device called: Accurate Test of Limb Isometric Strength (ATLIS). This portable device consists of an adjustable tilting chair in a frame. A fixed wireless load cell measures MVIC of 12 limb muscle groups, tested in standard, gravity-eliminated positions. The testing protocol takes 15 minutes to complete.

Objectives: 1) To determine the test-retest and inter-rater reliability and the criterion-based validity of the ATLIS protocol; 2) To determine subject acceptance of this new testing protocol.

Methods: To determine test-retest reliability of the ATLIS protocol, 20 healthy adults and 10 subjects with ALS were tested twice by the same evaluator. Inter-rater reliability was determined by testing 20 healthy adults and 10 subjects with ALS twice: once by each of two evaluators. Concordance of each item was determined by calculating the mean absolute percent difference between the paired tests. To determine criterion-related validity, 20 healthy adults were tested using the ATLIS protocol and also using a well-validated protocol: Tufts Quantitative Neuromuscular Exam (TQNE).
Subjects were also asked to complete a five item survey using a Likert scale to assess their acceptance of the ATLIS protocol.

Results: The sample of healthy adults consisted of a 2/3 ratio of females to males with a mean age of 36 years. The sample of subjects with ALS was roughly equally distributed between males and females with an average age of 57 years. Mean absolute differences between tests ranged between 6 and 11% in all muscle groups. Comparisons between ATLIS and TQNE values of the 12 muscles groups demonstrated acceptable correlations (0.44 - 0.92), though TQNE values were higher than ATLIS values. Over 95% of ALS subjects reported agreement or strong agreement regarding the attributes of ATLIS.

Discussion and conclusions: Reliability studies indicate that reliability of ATLIS is equal to or better than the strain gauge or HHD testing. Test-retest reliability was only slightly better than inter-rater reliability indicating that perhaps subject performance accounted for the majority of variance rather than differences between the evaluators. Though the correlations of ATLIS and TQNE scores were acceptable, the higher scores seen with TQNE may be due to differences in testing positions. The survey of ATLIS attributes indicated overwhelming acceptance by subjects with ALS.
ATLIS shows great promise to become an efficient and precise outcomes measure for future clinical trials in ALS.

C31 MUSCLE ULTRASONOGRAPHY AS A DIAGNOSTIC TOOL FOR ALS: A DIAGNOSTIC STUDY ACCORDING TO THE STARD CRITERIA

ARTS I[1], PILLEN S[1], ZWARTS M[1], BOEKESTEIN W[1,2], KLEINE B[1], OVEREEM S[1], SCHELHAAS HJ[1]

[1]*Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [2]*Medical Spectrum Twente, Enschede, Netherlands*

E-mail address for correspondence: IM.Arts@neuro.umcn.nl

Keywords: ultrasonography, diagnosis, STARD

Background: Ultrasonography can detect signs of lower motor neuron (LMN) loss, by visualizing fasciculations, diminished muscle thickness and increased echo intensity. Here, we evaluated the diagnostic potential of ultrasonography in ALS, in a prospective study according to the STARD criteria.

Objectives: To study the diagnostic value of ultrasonography in differentiating between ALS/SMA and mimics. Furthermore, to assess the ability of ultrasonography to detect LMN signs in clinical unaffected regions and thereby increasing the diagnostic certainty of ALS.

Methods: Fifty-nine patients, recruited from our MND clinic, received a diagnostic work up according to the standards of the revisited El Escorial criteria. In addition, bilateral transverse ultrasonography scans were made of the sternocleidomastoid, biceps, forearm flexors, rectus abdominis, quadriceps and tibialis anterior muscles. Muscle thickness and echo intensity were quantitatively measured. Each muscle was screened for fasciculations during 10 seconds. Ultrasonography was considered indicative for ALS/SMA when echo intensity was $\geq$ 1.5 SD above normal in at least 2 muscles and fasciculations were present in $\geq$ 4 muscles. In patients with ALS, we evaluated each region separately in order to quantify the number of affected regions. Requirements for ultrasonographic regional LMN involvement were: fasciculations in $\geq$ 1 one muscle for the bulbar and thoracic region, and fasciculations $\geq$ 2 muscles for the cervical and lumbosacral regions.

Results: Ultrasonography was able to differentiate between ALS/SMA and mimics with 96% sensitivity and 84% specificity. In the 24 ALS patients, ultrasonography detected 13 regions with LMN involvement that were negative using either clinical examination or needle EMG. In 10 of the 12 patients with clinical possible ALS, ultrasonography detected LMN involvement in $\geq$ 3 regions. The remaining two patients did not fulfil the EMG criteria for probable ALS laboratory-supported.

Discussion: Muscle ultrasound is able to differentiate between ALS/SMA and mimics with high sensitivity and specificity. In addition, non-invasive ultrasonography is superior as compared to needle EMG to detect additional affected regions in patients diagnosed with ALS. Especially the sensitivity to detect fasciculations is an important asset of ultrasonography.

Conclusion: Our findings warrant the incorporation of ultrasonography in the diagnostic work up of ALS.

C32 A NOVEL CMAP SCAN-BASED PROGRESSION SCORE FOR MOTOR NEURON DISEASE

MAATHUIS E, DRENTHEN J, VAN DOORN P, VISSER G, BLOK J

Erasmus Medical Center, Rotterdam, Netherlands

E-mail address for correspondence: e.maathuis@erasmusmc.nl

Keywords: CMAP scan, progression, neurophysiology

Background: Motor neuron disease (MND) is marked by ongoing motor unit (MU) loss, reinnervation, and ultimately muscle atrophy. The effects of these three processes on existing markers of disease progression partly counteract each other (reinnervation masks MU loss). Hence, electrophysiological features of disease progression can only be monitored accurately if all three processes are taken into account. In the present study, we propose the first neurophysiological marker that combines quantitative information on the number of surviving MUs, reinnervation, and the extent of muscle atrophy: the CMAP scan progression score (CSPS). Each CSPS is derived from a CMAP scan, a novel electrophysiological assessment that can be performed easily and noninvasively in less than 10 minutes. The CMAP scan is basically a high-detail stimulus-response curve (normally smooth and sigmoid in shape), in which the results of reinnervation processes appear as so-called steps ('gaps' in the curve). Changes in the number and size of steps reflect the underlying process of loss of functioning MUs. The muscle fiber mass (reduced in atrophy) is expressed through the maximum CMAP amplitude.

Objective: To determine the value of the CSPS as a quantitative electrophysiological marker of disease progression in MND.

Methods: CMAP scans were recorded five times with a 2-3 week interval from the thenar muscles of 10 MND patients. After these 5 recordings at regular intervals, 8 patients had 1 to 8 additional recordings over a total follow up period that extended up to 85 weeks (median 34 weeks). Motor unit number estimation (MUNE) was performed in the same sessions for comparison.
The CSPS is the sum of 3 subscores: the reinnervation score (decreases with increase in step size, 1-5 points), the atrophy score (decreases with decrease in CMAP amplitude, 1-5 points) and the motor unit number score (based on average step size; decreases with increasing step size, 1-4 points). The total CSPS ranges from 14 (no change) to 3 (severe deterioration). To compare variables between days, paired nonparametric tests were used.

Results: Median CSPS on day 1 was 11.5 (range 9-14). Over the first 5 recordings, 9/10 (90%) of the patients showed a decrease in CSPS; median CSPS at the fifth recording was 11 (range 6-14) ($P = 0.03$). MUNE and the maximum CMAP amplitude did not differ significantly between the first and fifth measurements. During long-term follow up, CMAP amplitude, MUNE, and CSPS decreased in all patients.

Discussion and conclusions: The CSPS is the first electrophysiological MND marker that combines the effects of MU loss, reinnervation and atrophy. It can be obtained with a simple, brief, and non-invasive test. Furthermore, the CSPS is objective, informative, easy to interpret and appears to represent disease progression more accurately than MUNE or CMAP amplitude.

C33 FURTHER EVIDENCE FOR MULTISYSTEM INVOLVEMENT IN ALS: MULTIPARAMETRIC MRI OF SENSORY STIMULATION

LULÉ D[1,2], DIEKMANN V[1], MÜLLER H-P[1], KASSUBEK J[1], BIRBAUMER N[2,3], LUDOLPH AC[1]

[1]*Department of Neurology, University of Ulm, Ulm, Germany,* [2]*Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Tübingen, Germany,* [3]*Ospedale San Camillo, IRCCS, Instituto di Ricovero e Curo a Carattere Scientifico Venezia, Venice, Italy*

E-mail address for correspondence: dorothee.lule@uni-ulm.de

Keywords: sensory system, fMRI, DTI

Background: In recent years, evidence for cortical changes in amyotrophic lateral sclerosis (ALS) beyond the motor cortex has accumulated. Modern imaging techniques bear the potential to further investigate neurodegeneration and reorganisation in the cortex of ALS patients.

Objectives: Structural and functional imaging techniques were combined to investigate sensory system function in ALS.

Methods: Functional magnetic resonance imaging (fMRI) was used to investigate cortical activity during visual, auditory, and somato-sensory stimulation in fourteen ALS patients and eighteen control subjects. Changes in amplitude, latency and duration of the BOLD response were modelled. Furthermore, diffusion tensor imaging was used to investigate changes in white matter networks.

Results: During visual stimulation, fMRI demonstrated a decreased response in secondary visual areas in ALS possibly related to functional deficits of sensory nerve fibres. The stronger the decrease in physical functioning (measured with ALS-FRS) the higher was the brain activity in associative cortices. This might represent a compensatory process. Additionally, reduced functioning became evident for fibres projecting to extra-striate visual cortex.
For auditory stimulation, a delayed response in secondary auditory areas probably linked to prolonged conductance or synaptic transmission times and an altered cortical pattern in areas involved in target processing/detection became evident in ALS patients. Structural white matter differences in the primary and secondary auditory cortices were observed in ALS patients compared to controls. For somato-sensory stimulation a prolonged/reduced response in sensory integration areas of the parietal lobe was observed perhaps linked to the reduced visceral inflow due to immobility.

Discussions: Multiparametric MRI suggests a progressive functional deficit in secondary/higher order sensory processing areas in ALS. Accordingly, data provide evidence for a primary pathology of the sensory system in ALS highlighting the multisystematic character of the disease. Evidence for compensatory processes in multimodal associative cortices was found. This might be an expression of a general capacity for cortical plasticity in severe neurological disorders.

Conclusion: The present study provides evidence for structural and functional changes and reorganisation in the sensory cortex of ALS patients. Our data adds essential keynotes to the understanding of the multisystematic character of the disease ALS.

C34 BASELINE CORTICOSPINAL TRACT MRI REFLECTS CHANGE IN DISABILITY AT SIX MONTH FOLLOW-UP

ABRAHAM I, MENKE R, FILIPPINI N, TALBOT K, TURNER M

Oxford University, Oxford, United Kingdom

E-mail address for correspondence: martin.turner@clneuro.ox.ac.uk

Keywords: diffusion tensor imaging, prognosis, biomarker

Background: The diagnostic pathway, prognostication and therapeutic trial monitoring could all be improved by the establishment of robust biomarkers in ALS. Diffusion tensor imaging (DTI) is a particularly promising application of MRI, with the ability to detect white matter (WM) damage. DTI has demonstrated consistent cerebral WM involvement in ALS within (though not limited to) the corticospinal tract (CST). There would be clear value in a non-invasive MRI marker of WM damage that could predict future disability, which might then be applicable to therapeutic trials.

Objectives: To identify whether regional baseline measurement of CST WM integrity, as assessed by DTI, reflects disease progression at six months.

Methods: Twelve ALS patients at various stages of disease underwent cerebral MRI at high-field (3T) with a six-month interval between the two studies. Fractional anisotropy (FA) maps were generated using tools from FMRIB Software Library (FSL). Tract-based spatial statistics (TBSS) were performed within the left and right CST in MNI space to assess voxel-wise correlations of DTI indices with the difference between follow-up and baseline ALSFRS-R scores. Results were considered significant at $P < 0.05$ (corrected for multiple comparisons, FWE) using threshold-free cluster enhancement (TFCE).

Results: A highly significant ($P = 0.002$) negative correlation was noted between baseline FA values and change in ALSFRS-R score after six months, for a region spanning the internal capsules of the CST bilaterally.

Discussion: CST FA reduction is accepted to reflect the degree of WM involvement in ALS, and its baseline correlation with change in disability after six months in a heterogeneous group of patients suggests it may have prognostic potential. This must be explored further in larger studies with longer follow up and multivariate survival analysis.

Conclusions: DTI has clear potential to provide biomarkers that may be sensitive to both prognosis and therapeutic monitoring in ALS. Consideration should be given to cerebral DTI as a secondary outcome measure in future therapeutic trials to fully realise this potential.

C35 DIAGNOSTIC BIOMARKERS FOR ALS

GANESALINGAM J[2], SHAW C[2], SHAW G[3], LACOMIS D[1], BOWSER R[1]

[1]*University of Pittsburgh School of Medicine, Pittsburgh, PA, United States,* [2]*MRC Centre for Neurodegeneration Research, Kings College London, United Kingdom,* [3]*University of Florida, Gainesville, FL, United States*

E-mail address for correspondence: bowserrp@upmc.edu

Keywords: biomarkers, mass spectrometry, cerebrospinal fluid

Background: Diagnostic biomarkers for ALS are desired to shorten the time between symptom onset and patient diagnosis. Diagnostic biomarkers may also be used to stratify the ALS patient population and potentially identify sub-populations that may best respond to specific treatments. Prior studies have identified a number of candidate biomarkers in the cerebrospinal fluid (CSF). We have further explored a specific set of proteins as diagnostic biomarkers for ALS within the CSF and plasma.

Objectives: To evaluate the diagnostic utility of neurofilament and complement c3 proteins as biomarkers for ALS.

Methods: CSF was obtained from 163 subjects and used for ELISA to measure levels of phosphorylated neurofilament heavy chain (p-NFH) and complement c3 proteins. The sample set was 71 ALS patients (median disease duration of 16 months from symptom onset), 40 healthy controls, and 52 disease controls that included 14 disease mimics. A training set of 106 and a test set of 57 CSF samples were generated from these samples. ROC curves of the training set were used to generate cut-off values for each protein that were subsequently used to determine diagnostic utility in the test set.

Results: The median level of pNFH was 1.77 ng/ml, 0.2 ng/ml and 0.165 ng/ml for ALS, disease control (DC) and healthy control (HC) groups, respectively. A p-NFH cut-off level of 0.635 ng/ml generated a sensitivity of 84% sensitivity and 93% specificity for ALS in the training set. Complement c3 was also significantly increased in ALS versus HC and DC versus HC groups, but not ALS versus DC. This was likely due to inflammation within the various members of the DC group. For complement c3, a threshold value of 3.62 μg/ml produced a sensitivity of 62% and specificity of 56% for ALS. However a ratio of p-NFH to complement c3 was found to provide the best overall diagnostic accuracy. A p-NFH/complement c3 ratio of greater than 0.000125 provided 91% sensitivity and 89% specificity for the training set. When this p-NFH to complement c3 ratio was applied to the test set, we obtained a sensitivity of 96% and specificity of 90%.

Conclusions: Levels of p-NFH and complement c3 are elevated in the CSF of ALS and other neurologic disorders. However we identified a specific ratio of the two proteins that provided over 90% diagnostic accuracy in CSF from 163 subjects. We also correlated CSF levels of p-NFH to plasma levels within the same subjects. Our results suggest that ELISA measurements for these two proteins within the CSF may provide a diagnostic predictor of ALS. Further studies are required to validate these results.

SESSION 6A TRANSLATIONAL STRATEGIES

C36 PLASTIC VIRUSES: ENGINEERING NANOPARTICLES FOR TARGETING THE CENTRAL NERVOUS SYSTEM

BATTAGLIA G

The Krebs Institute, Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom

E-mail address for correspondence: g.battaglia@sheffield.ac.uk

Keywords: bionanotechnology, synthetic technology, delivery system

Provided the right hydrophilic/hydrophobic balance can be achieved, amphiphilic block copolymers are able to assemble in water into membranes. These membranes can enclose forming spheres with an aqueous core. Such structures, known as polymer vesicles or polymersomes (from the Greek -some = body of), have sizes that vary from tens to thousands of nanometers. The wholly synthetic nature of block copolymers affords control over parameters such as the molar mass and composition, which ultimately determine the structure and properties of the species in solution. By varying the copolymer molecular mass it is possible to adjust the mechanical properties and permeability of the polymersomes, while the synthetic nature of copolymers allows the design of interfaces containing various biochemically-active functional groups (1). In particular, non-fouling and non-antigenic polymers have been combined with hydrophobic polymers in the design of biocompatible nano-carriers that are expected to exhibit very long circulation times. Stimulus-responsive block copolymers have also been used to exploit the possibility to trigger the disassembly of polymersomes in response to specific external stimuli such as pH, oxidative species, and enzyme degradation (1). Such bio-inspired bottom-up supramolecular design principles offer outstanding advantages in engineering structures at a molecular level, using the same long-studied principles of biological molecules. Thanks to their unique properties, polymersomes have already been reported and studied as delivery systems for both drugs, genes, and image contrast agents as well as nanometer-sized reactors (1).

In the recent years we have studied and optimized the molecular parameters that control polymersomes assembly as well as indentified several formulation of their ad hoc disassembly (2–5). We are able to control the shape and the surface morphology of the polymersomes at the nanoscale (6). We are now using these molecular engineering tools to design nanoscopic vectors for the effective and targeted delivery of therapeutic and image contrast agents. We have particularly demonstrated that by formulating pH sensitive polymersomes we can achieve high cytosolic delivery of small molecules as well as large macromolecules like nucleic acids and proteins (7–9).

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C37 APPLYING IPSC TECHNOLOGY FOR MOTOR NEURON DISEASE: A PATIENT FIRST DRUG DISCOVERY PLATFORM

STRULOVICI B, JAVAHERIAN A

iPierian, San Francisco, CA, United States

E-mail address for correspondence: berta.strulovici@ipierian.com

Keywords: iPSC, neurons, pharmaceuticals

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with complex genetic and environmental contributors. The current standard drug discovery platform for human disease typically relies on some knowledge about the molecular mechanism of the disease and a target in mind. However, for complex diseases such as ALS where the molecular mechanism underlying disease pathology is not well understood, target-based biochemical assays are, at best, speculative or not feasible. Due to the lack of predictive *in vivo* and *in vitro* models that represent sporadic as well as familial ALS, traditional drug discovery strategies using surrogate cell lines and animal models have not been successful for ALS. We have challenged the traditional drug discovery approaches to this disease by putting the patient at the forefront of drug discovery. Induced pluripotent stem cell (iPSC) technology allow us, for the first time in the history of drug discovery, to study neurologic disorders in patient-derived neural cells. We are using iPS cell lines from both familial and sporadic ALS patient fibroblasts differentiated into motor neurons and glia for identification of ALS disease phenotypes. We have industrialized the process of reprogramming patient cells to produce large numbers of neurons and glia, the cell types implicated in ALS, in a highly robust and reproducible manner. We are applying disease phenotypes discovered in patient motor neurons and glia in high throughput assays to screen libraries of compounds to identify therapeutic agents for ALS. We are executing this project in a scale large enough to enable testing of compounds across a large number of patient lines that represent different forms of ALS in a novel and innovative '*in vitro* clinical trial' approach. The discovery of a novel therapeutic compound for ALS could have a major impact on over 25,000 patients in the United States. More importantly, if successful, our new drug discovery platform could be applied to a variety of complex diseases where animal and simple cell models are not adequate to address the complexities of the human population.

SESSION 6B EPIDEMIOLOGY

C38 CAN WE IDENTIFY RISK FACTORS FOR ALS/MND?

BEGHI E

Istituto Mario Negri, Milano, Italy

E-mail address for correspondence: ettore.beghi@marionegri.it

Keywords: epidemiology, risk factors

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder with worldwide distribution. The geographical distribution of ALS is fairly homogeneous except for the Western Pacific variant of the disease (the ALS/parkinsonism dementia complex) formerly thought to occur at a 100th fold rate. This significant increase in the risk of ALS in the local populations has been attributed, among others, to the cycad hypothesis, according to which ALS may be caused by a neurotoxic aminoacid, beta-methylaminoalanine (BMAA) present in a palm seed (the Cycad micronesica). Recent discoveries found that BMAA is produced by symbiotic cyanobacteria within the cycad roots and that animals forage on the palm's seeds, bringing the toxin into the food chain. This brings the attention back to the role of environmental factors as possible causes of ALS. In the western countries several occupational, alimentary and other environmental factors have been investigated with disappointing results. In fact, the assessment of the environmental risk factors in ALS is based on a high number of observations of uncertain etiological significance. These include conjugal ALS, correlation with antecedent poliomyelitis or concurrent neoplasms, exposure to (heavy) metals, to solvents or to electrical or electromagnetic fields, mechanical trauma, heavy physical activity, and living in rural areas or using chemical substances in agriculture. The inconsistent findings can be explained in light of the poor methodology of most published reports. The inclusion of non-representative study samples, the lack of standard definitions for exposures, and the small sample size are among the commonest methodological defects. The use of prevalent rather than incident cases is a source of selection bias. Differing definitions of putative risk factors prevent meaningful comparisons across studies to verify the consistency of the results. As the disease is rare and also several exposures are uncommon in the general population, most case-control studies have been insufficiently powered to detect significant differences in the rate of exposure among ALS patients and matched controls. In this regard, only large population-based case-control studies done in incident ALS patients and using standard definitions for exposures are proper tools to investigate risk factors for ALS.

C39 EPIDEMIOLOGY OF ALS IN THE NETHERLANDS, 2006-2009

HUISMAN MHB[1], DE JONG SW[1], VAN DOORMAAL PTC[1], WEINREICH S[2], SCHELHAAS HJ[3], VAN DER KOOI AJ[4], DE VISSER M[4], VELDINK JH[1], VAN DEN BERG LH[1]

[1]*Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Dutch Neuromuscular Patient Association, Baarn, Netherlands,* [3]*Department of Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [4]*Department of Neurology, Amsterdam Medical Center, Amsterdam, Netherlands*

E-mail address for correspondence: m.h.b.huisman-3@umcutrecht.nl

Keywords: epidemiology, population-based, incidence

Background: Prior population-based registries of ALS are limited by small population size and no adjustment for differential coverage of patients in different age and sex classes by making an estimate of the number of unobserved patients. Due to these limitations it is not clear whether the reported incidence rate decline in the very elderly and the decreased male to female ratio in postmenopausal age classes are real.
Shared exposure to an environmental risk factor might result in geographical clustering of ALS. Identification of these clusters could be a starting point to discover new exogenous risk factors.
Further clues for environmental and genetic risk factors could be retrieved by investigating the family history of ALS patients, since relatives share genetic and environmental risk factors.

Objectives: 1) To provide reliable data on ALS epidemiology; 2) To identify geographical clusters of ALS; 3) To determine whether the occurrence of ALS, Parkinson's Diseases (PD), dementia and vascular diseases in relatives of patients with ALS is different from the occurrence in relatives of controls.

Methods: A population based study has been performed in the Netherlands between January 2006 and December 2009 (mean population 16,426,273; area 41,528 km^2). Patients were ascertained from five sources. Diagnosis was made according to the El Escorial criteria. Capture-recapture analysis in each 5 year age group for both genders apart was performed to correct incidence and prevalence rates for the estimated number of missing patients. Spatial scanning software was applied to test for geographical clustering by using residential zip code at diagnosis and 5 years before diagnosis. 635 patients and 1616 age- and sex-matched controls filled in questionnaires concerning the diagnosis of PD, dementia, myocardial infarction (MI) or stroke in first-, second- and third-degree relatives.

Results: The incidence rate of ALS was 2.77 per 100,000 person-years (95% CI 2.76-2.79). Prevalence rate at 31 December 2008 was 10.32 per 100,000 people (95% CI 10.27-10.37). Both incidence and prevalence peaked in the 70-74 years age group. Although the male: female incidence rate ratio in the premenopausal age group was higher than in the postmenopausal age group, 1.89 and 1.50 respectively, this difference was not statistically significant.
Two geographical clusters of ALS were identified; one with a relative risk of 3.1 (radius 15 kilometers, P-value 0.015) and one

with a relative risk of 1.8 (radius 27 kilometers, P-value 0.034).
Relatives of patients have an elevated risk of ALS compared to controls (λ 2.22; 95% CI 2.16-2.26). Dementia (λ 1.14; 95% CI 1.09-1.18) and PD (λ 1.13; 95% CI 1.03-1.23) are more common among relatives of ALS patients compared with relatives of controls. The occurrence of stroke (λ 0.93; 95% CI 0.88-0.97) and MI (λ 0.84; 95% CI 0.80-0.88) in relatives of ALS patients is lowered.

Discussion and conclusions: We report the epidemiology of ALS in the largest individual population based register until now. Application of the capture recapture method confirmed that incidence rates of ALS decrease after age 74 years. Further, it showed that the previously found postmenopausal decrease in the male to female ratio is possibly a result of bias. Two significant clusters were detected which need further investigation and may provide clues to the etiology of MND. The results on the family history of ALS support an association between neurodegenerative diseases and ALS, indicating that these diseases share genetic and environmental risk factors. The lowered risk of vascular diseases in relatives of ALS patients may suggest that a beneficial vascular risk profile increases the susceptibility for ALS.

C40 ALS PHENOTYPIC HETEROGENEITY: EVIDENCE FROM A POPULATION-BASED STUDY

CHIO A[1], CALVO A[1], MAZZINI L[2], MORA G[3], BOTTACCHI E[4], BALMA M[1], GALLO S[1], CANOSA A[1], MUTANI R[1]

[1]*Department of Neuroscience, University of Torino, Torino, Italy,* [2]*Department of Neurology, University of Eastern Piedmont, Novara, Italy,* [3]*Department of Neurorehabilitation, IRCSS Salvatore Maugeri, Milano, Italy,* [4]*Regional Hospital, Aosta, Italy*

E-mail address for correspondence: achio@usa.net

Keywords: phenotypes, incidence, outcome

Aim: The epidemiological and clinical characteristics of ALS phenotypes in the patients included in an Italian prospective epidemiological register have been assessed.

Background: The characteristics of ALS clinical phenotypes (bulbar, classical (Charcot), pyramidal, flail leg, flail arm and respiratory) remain poorly understood. Moreover, no studies have been performed on these phenotypes in an epidemiological setting.

Methods: The patients prospectively diagnosed and followed-up between 1995 and 2004 in Piemonte and Valle d'Aosta have been classified according to their clinical phenotype. The effect of the phenotypes on ALS prognosis has been also analyzed.

Results: Of the 1260 incident patients, 1241 (98.5%) had complete phenotypic data. The most common phenotype was bulbar ALS (mean incidence rate 1.1/100,000/year, with no difference between genders) and the second was classical ALS (incidence rate, 1.2 men and 0.9 women; men to women rate ratio 1.7:1). Flail leg syndrome and pyramidal phenotype had a similar frequency in both genders (incidence rate, 0.4 and 0.3, respectively). Flail arm syndrome and respiratory phenotype were largely more represented among male (men to women rate ratio 4.0:1 and 9.0:1, respectively). The oldest age at onset was found in the bulbar phenotype (68.8 years) and the lowest in the pyramidal phenotype (58.3 years). Frontotemporal dementia was more frequent in bulbar phenotype (9.0%) and very rare in the flail arm syndrome (1.4%). Significantly different outcomes were found: pyramidal and flail arm phenotypes had the better prognosis (median survival, 6.3 and 4.0 years, respectively), while bulbar and respiratory phenotypes had the worst prognosis (2.0 and 1.4 years, respectively).

Conclusions: ALS phenotypes are largely related to a complex interplay between gender and age. The reasons for the strong influence of these factors on ALS biology remains largely unknown. In turn, ALS phenotypes are the amongst the main factors determining the clinical outcome.

SESSION 8A GENETICS AND GENOMICS

C41 CLINICAL, PATHOLOGICAL AND GENETIC FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS ASSOCIATED WITH OPTINEURIN MUTATIONS

KAMADA M[1], IZUMI Y[1], ITO H[2], MARUYAMA H[3], KUSAKA H[2], MORINO H[3], KAWAKAMI H[3], KAJI R[1]

[1]*Tokushima University, Tokushima, Japan,* [2]*Kansai Medical University, Osaka, Japan,* [3]*Hiroshima University, Hiroshima, Japan*

E-mail address for correspondence: kajkyoto@mbox.kyoto-inet.or.jp

Keywords: Optineurin, ALS, NFkB

We have recently reported mutation of the gene of Optineurin (OPTN), an inhibitor of NFkB, in ALS. Although the clinical features fulfilled the El Escorial criteria (laboratory-supported probable), 2 families (heterozygous E478 mutation) out of 5 exhibited slow clinical progression (~ 8 years without ventilation) with clinically symmetric onset. Another family with homozygous exon 5 deletion showed early and asymmetric onset and more rapid progression (intubated 4 years after onset). Two families with homozgygous Q398X mutation showed intermediate course between these. The phenotypic difference might be correlated with the site of mutation within OPTN. Because abnormal accumulation of OPTN in motor neurons was found not only in those with OPTN mutation but also SOD1 positive and sporadic ALS patients, NFkB pathway may have a pivotal role in ALS. The phenotypic difference among OPTN positive patients should give insights into the specificity and efficacy of NFkB inhibitors for the development of possible therapeutic agents for ALS in general.

C42 A LARGE CNV ASSOCIATION STUDY IN SPORADIC ALS

BLAUW H[1], AL-CHALABI A[3], ANDERSEN P[4,5], VAN VUGHT P[1], VAN ES M[1], WAIBEL S[5], MEYER T[8], HARDIMAN O[9], TOMIK B[11], SLOWIK A[11], BROWN R[10], LANDERS JE[10], ROBBERECHT W[14], LUDOLPH AC[5], OPHOFF R[2,17], VELDINK JH[1], VAN DEN BERG LH[1]

[1]*Department of Neurology, UMC Utrecht, Utrecht, Netherlands,* [2]*Department of Medical Genetics and Rudolf Magnus Institute, Utrecht, Netherlands,* [3]*Department of Clinical Neuroscience, King's College, London, United Kingdom,* [4]*Department of Clinical Neuroscience, Umeå University, Umeå, Sweden,* [5]*Department of Neurology, University of Ulm, Ulm, Germany,* [6]*Department of Internal Medicine, Erasmus University Medical Centre Rotterdam, Rotterdam, Netherlands,* [7]*Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [8]*Department of Neurology, Charité University Hospital, Humboldt-University, Berlin, Germany,* [9]*Department of Neurology, Beaumont Hospital, Dublin, Ireland,* [10]*Department of Neurology, Massachusetts General Hospital, Charlestown, United States,* [11]*Department of Neurology, Jagiellonian University, Krakow, Poland,* [12]*Experimental Neurology, University of Leuven, Leuven, Belgium,* [13]*Vesalius Research Centre, Flanders Institute for Biotechnology (VIB), Leuven, Belgium,* [14]*Department of Neurology, University Hospital Leuven, University of Leuven, Leuven, Belgium,* [15]*Division of Molecular and Clinical Neurobiology, Department of Psychiatry, Ludwig-Maximilians-University, Munich, Germany,* [16]*Department for Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, and German Center for Neurodegenerative Diseases, Tuebingen, Germany,* [17]*UCLA Center for Neurobehavioral Genetics, Los Angeles, United States*

E-mail address for correspondence: h.m.blauw@umcutrecht.nl

Keywords: genome-wide association study, CNV, rare variant

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease selectively affecting motor neurons in brain and spinal cord. Recent genome-wide association studies have identified several common variants (SNPs) that increase disease susceptibility. In contrast, rare copy-number variants (CNVs), which have been associated with several neuropsychiatric traits, have not been studied for ALS in well-powered study populations.

Objectives: To identify rare CNVs that increase disease susceptibilty in sporadic ALS.

Methods: We conducted a two-staged association study focused on rare CNVs, including over 19,000 individuals. We analysed genome-wide Illumina data and DNA samples using stringent quality control criteria. We used PennCNV software for CNV detection and applied extra quality control filters to reduce the number of false-positive CNV calls. We tested each gene for association by comparing the number of CNVs affecting the gene in cases versus controls. Loci (genes) with a nominal Fisher Exact P value < 0.01 and a frequency of $<1\%$ in controls were selected for follow-up after careful validation with TaqMan qPCR.

Results: In the discovery cohort of 1,875 ALS patients and 8,731 controls we identified two loci that met our criteria for follow-up: the *DPP6* locus (CNVs in 10 of 1,875 ALS patients versus 13 of 8,731 controls, OR=3.59, $P=6.6 \times 10^{-3}$), and

the 15q11.2 locus, containing *NIPA1*, the gene causing hereditary spastic paraparesis (HSP) type 6 (CNVs in 8 of 1,875 ALS patients versus 3 of 8,731 controls, OR=12.46, $P=9.3\times10^{-5}$). Validation experiments confirmed the presence of all (n=25) tested CNVs.
In our replication population of 2,559 ALS patients and 5,887 controls, the genes of interest again contained more CNVs in patients compared to controls, but did not meet our criteria for independent replication: *DPP6* locus: 10 CNVs in 2,559 ALS patients versus 12 in 5,887 controls, OR=1.92, P=0.097, pooled results: OR=2.64, $P=1.4\times10^{-3}$; *NIPA1*: 7 CNVs in 2,559 ALS patients versus 5 in 5,887 controls, OR=3.23, P=0.041, pooled results: OR=6.20, $P=2.2\times10^{-5}$).

Discussion: We identified two genes that show suggestive evidence for association with ALS disease status. *DPP6* has been suggested previously as a candidate gene for ALS, while *NIPA1* has not been associated with ALS before. Mutations in *NIPA1* cause HSP type 6, a disease characterised by the selective death of (central) motor neurons, suggesting an important role in motor neuron biology. Statistical power remains a problem in studies aimed at rare variants.

Conclusions: CNV analyses provide suggestive evidence for *DPP6* and *NIPA1* as candidate susceptibility genes for ALS.

C43 RARE MUTATIONS IN ANGIOGENIN CONFER LARGE RISK FOR SPORADIC ALS

VAN ES M[1], VAN VUGHT P[1], SCHELHAAS H[2], FUMOTO K[1], ANDERSEN P[3], GROEN E[1], BLAUW H[1], DIEKSTRA F[1], CUPPEN E[1], LEMMENS R[4], VAN DAMME P[4], ROBBERECHT W[4], DAHLBERG C[3], DE VISSER M[5], VAN DER KOOI A[5], LUDOLPH AC[1], VELDINK JH[1], PASTERKAMP JR[1], OPHOFF R[1], VAN DEN BERG LH[1]

[1]*UMC Utrecht, Utrecht, Netherlands,* [2]*Radboud University Nijmegen Medical Center, Nijmegen, Netherlands,* [3]*Umeå University Hospital, Umeå, Sweden,* [4]*University Hospital Leuven, Leuven, Belgium,* [5]*Amsterdam Medical Center, Amsterdam, Netherlands,* [6]*University of Ulm, Ulm, Germany*

E-mail address for correspondence: m.a.vanes@umcutrecht.nl

Keywords: angiogenin, rare variation, association

Background: A large candidate gene study demonstrated an association between a SNP (rs11701) in *ANG* and sporadic ALS. Mutations in *ANG* were also reported in SALS patients. However, subsequent follow-up studies have unfortunately produced conflicting results. The association for the SNP could not be replicated and mutations were also identified in healthy controls.

Objectives: The aim of this study was to provide a definitive answer on the role of *ANG* in ALS.

Methods: The association for rs11701 was tested in a population of over 20,000 individuals. We performed a meta-analysis on all previous studies in *ANG* in ALS and performed additional sequencing experiments, allowing us to analyze sequence data from over 6,000 cases and 6,000 controls.

Results: Analysis of all data revealed a total of 16 rare non-synonymous mutations in 25 patients out of a total 6,006 sporadic ALS patients (0.42%). By comparison these variants were not observed in any of the 6,296 control subjects. Statistical analysis showed that this aggregation of rare variation in cases is significant with $P = 3.28 \times 10^{-7}$. Results were consistent across all populations and a subsequent Woolf test did not detect evidence for heterogeneity between the strata with P = 0.97. These rare variants indeed confer a greater effect on disease risk than the common polymorphisms identified by GWAS and thus partially explain the missing heritability. We found that the odds ratio (OR) for rare mutations in *ANG* is 27.37 with 95% confidence interval (95% CI) of 6.31 - 449.68.

Discussion: We show a significant excess of rare mutations in *ANG* in sporadic ALS patients compared to controls. These mutations confer a very significant risk for ALS with OR > 25.0.

Conclusions: Mutations in *ANG* confer large risk for ALS.

C44 PARAOXONASE GENE MUTATIONS IN AMYOTROPHIC LATERAL SCLEROSIS

TICOZZI N[1,2], LECLERC AL[2], KEAGLE P[2], GLASS JD[3], WILLS A-M[4], VAN BLITTERSWIJK M[2], BOSCO D[2], RODRIGUEZ-LEYVA I[5], GELLERA C[6], RATTI A[1], TARONI F[6], MCKENNA-YASEK DM[2], SAPP PC[2,7], SILANI V[1], FURLONG CE[8], BROWN RH[2], LANDERS JE[2]

[1]*Department of Neurology, Università degli Studi di Milano, IRCCS Istituto Auxologico Italiano, Milan, Italy,* [2]*Department of Neurology, University of Massachusetts Medical School, Worcester, MA, United States,* [3]*Department of Neurology, Emory University, Atlanta, GA, United States,* [4]*Department of Neurology, Massachusetts General Hospital, Charlestown, MA, United States,* [5]*Faculty of Medicine, Universidad Autonoma de San Luis Potosi, San Luis Potosi, Mexico,* [6]*Fondazione IRCCS Istituto Neurologico 'Carlo Besta', Milan, Italy,* [7]*Howard Hughes Medical Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, United States,* [8]*Department of Medicine, University of Washington, Seattle, WA, United States*

E-mail address for correspondence: n.ticozzi@fastwebnet.it

Keywords: genetics, paraoxonase, mutation

Background: The paraoxonase gene (*PON*) cluster on chromosome 7q21.3-q22.1 encodes for three homologous proteins (PON1, PON2 and PON3) involved in preventing lipid oxidation and detoxifying organophosphate compounds. Several reports have described an association between common SNPs in these three genes and sporadic amyotrophic lateral sclerosis (SALS) susceptibility.

Objectives: To assess whether mutations in the *PON* cluster are also involved in familial ALS (FALS) pathogenesis.

Methods: We screened for mutations in the *PON1*, *PON2* and *PON3* genes a cohort of 260 unrelated patients with a diagnosis of probable or definite ALS according to the El Escorial revised criteria and a positive family history for motor neuron disease. All patients were negative for *SOD1*, *TARDBP* and *FUS* mutations. Novel variants were genotyped in two panels composed of 1184 SALS and 1159 control samples.

Results: We identified 8 ALS-associated novel variants in the *PON* cluster in 12 samples (9 FALS and 3 SALS). All were heterozygous missense or splicing mutations, with the exception of a single homozygous mutation in *PON2*, identified in a patient whose parents were asymptomatic first cousins. The identified variants are predicted to disrupt protein function.

Discussion: Mutations in the *PON* cluster may contribute to ALS pathogenesis by impairing detoxification of exogenous toxins or by compromising the anti-oxidative capacity of the paraoxonase enzymes.

Conclusions: Our study suggests that mutations in the *PON* cluster may be responsible of ~2.5% of all FALS cases.

C45 JUVENILE ALS WITH BASOPHILIC INCLUSIONS IS A FUS PROTEINOPATHY WITH FUS MUTATIONS

BÄUMER D[1], BIBA A[1], HILTON D[2], PAINE S[3], LOWE J[3], TURNER M[1], ANSORGE O[1], TALBOT K[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Derriford Hospital, Plymouth, United Kingdom,* [3]*Nottingham University Medical School, Nottingham, United Kingdom*

E-mail address for correspondence: kevin.talbot@clneuro.ox.ac.uk

Keywords: FUS, basophilic inclusions, juvenile ALS

Background: The majority of cases of amyotrophic lateral sclerosis (ALS) start in late adult life and are characterised by TDP-43 positive ubiquitinated inclusions. Juvenile ALS is a rare but important exception with regard to age of onset and underlying neuropathology, which is TDP-43 negative, but characterised by basophilic inclusions on H+E stain.

Methods: We identified four patients with juvenile ALS with basophilic inclusions, analysed clinical features, characterised the neuropathology by immunohistochemistry and performed genetic and *in vitro* functional analyses, expressing FUS mutants in cell culture.

Results: Motor symptoms began between age 17 and 22, with rapid disease progression without dementia. No family history was identified. Basophilic inclusions were present in all cases and were immunoreactive for various RNA binding proteins, most prominently FUS (fused in sarcoma), but negative for TDP-43. A wide range of granular and compact FUS deposits was identified in glia and neuronal cytoplasm and nuclei. This was accompanied by disintegration of Nissl substance, ultrastructurally in keeping with aggregation of fragmented rough endoplasmic reticulum. Although no patient had a family history of motor neuron disease, genetic analysis revealed the presence of *FUS* mutations in all three cases with available DNA. Two patients carried apparent de novo P525L mutations. Post-mortem analysis and functional studies in cell culture indicate that these mutants may act by disrupting RNA binding protein complexes in the cytoplasm.

Conclusion: Juvenile ALS with basophilic inclusions is a FUS proteinopathy with features of endoplasmic reticulum disintegration. Most if not all cases are caused by mutations in *FUS*, suggesting that altered RNA metabolism is pivotal in disease pathogenesis.

SESSION 8B COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

C46 SOCIAL COGNITION IN ALS: IMPAIRED COGNITIVE AND AFFECTIVE THEORY OF MIND

VAN DER HULST E-J, BAK TH, ABRAHAMS S

University of Edinburgh, Edinburgh, United Kingdom

E-mail address for correspondence: s.abrahams@ed.ac.uk

Keywords: social cognition, cognitive change, prefrontal dysfunction

Background: A key component of Social Cognition is Theory of Mind (ToM) conceptualized as the ability to ascribe mental states to others. The Eye Gaze Test (EGT) is a classical ToM test and assesses an individual's ability to judge the preference of another by using the direction of their eye gaze as a cue. This process is fundamental for appropriate social interaction. Deficits on this test have been found in both ALS and FTD. ToM has been fractionated into cognitive and affective components, involving the recognition of the thoughts and feelings of another respectively. Disruption of affective ToM has been related to damage to the ventromedial prefrontal cortex, a region implicated early in FTD but which has not been as yet identified as typically affected in ALS.

Objectives: This study examined whether ALS patients display changes in both cognitive and affective ToM. Furthermore, the influence of attentional factors were explored.

Methods: Sixteen patients with ALS (non-demented) and 16 healthy participants, matched for age, sex and years of education, completed the cognitive and affective EGT. The EGT comprised of three features: type of judgement (cognitive, affective or physical control), attention (presence or absence of a distracter) and complexity of judgement (first or second order ToM). In addition, each participant was administered two tests of visual processing.

Results: The analyses revealed that ALS patients were significantly impaired in *both* cognitive and affective ToM trials. ALS patients displayed significantly lower scores for first order cognitive ($P < 0.01$) and affective judgements ($P < 0.005$) as well as second order cognitive judgements ($P < 0.05$) with a trend for second order affective judgements ($P = 0.067$). The patients were not impaired on physical control judgements or visual processing. Moreover there was no effect of having a distracter present.

Discussion and conclusions: Cognitive and affective components of ToM appear to be affected in some non-demented ALS patients who show difficulties in recognizing the thoughts *and feelings* of another. This deficit indicates involvement of the ventromedial prefrontal cortex and suggests that the prefrontal involvement in ALS may be extensive in those who do not show overt dementia. This selective deficit was found in patients with intact visual processing abilities. Moreover there was no effect of distracter present indicating that this was not the result of an attentional dysfunction. Hence this deficit reflects a basic social cognition impairment. These results could account for some of the behavioural abnormalities observed in ALS and implies that some ALS patients may have difficulties in primary interactions with carers.

C47 THE NATURE OF LANGUAGE DEFICITS IN ALS: SEMANTIC IMPAIRMENTS IN ACTION SEQUENCING

VAN DER HULST E-J, ABRAHAMS S, BAK TH

University of Edinburgh, Human Cognitive Neuroscience, Edinburgh, United Kingdom

E-mail address for correspondence: dina.jozefa@ed.ac.uk

Keywords: neuropsychology, language, embodied cognition

Background: A subset of patients with ALS show subtle difficulties in a range of cognitive functions, including language. In particular, recent research has uncovered deficits in action processing in ALS-patients both with and without dementia, although the exact nature of these deficits remains to be elucidated. Findings from these studies have been interpreted as support in favour of the influential theory of embodied cognition, which states that both motor functions and knowledge of actions are dependent on the same underlying neural networks.

Objectives: This study investigated the nature of linguistic and conceptual deficits in patients with classical ALS. In particular, it explored the distinction between two types of relationship between actions: parallel (actions of a similar character, eg *typing - writing*) and sequential (actions, which usually follow each other, eg *peeling - cutting*).

Methods: Twenty-one ALS-patients and 17 healthy controls (HCs), matched for age, education and sex, were compared on a neuropsychological battery of tests. The patients had to choose the correct answer from an array of two different choices. The battery comprised of language tests: semantic association for Objects and Actions (O and A) and Action Sequencing (AS). Furthermore, a relatively difficult test of Audio Visual Information Processing (AVIP) was added as a measure of complex non-semantic information processing. Both reaction times (RTs) and error rates were recorded.

Results: Analyses revealed that ALS-patients made significantly more errors than HCs on AS ($P = 0.021$), while both groups displayed an equal amount of errors on O and A ($Ps > 0.067$). In terms of the reaction times, taking out the possible influence of motor slowing, basic motor speeds were subtracted in all three conditions, yielding cognitive decision times. Results showed a main effect of group ($P = 0.016$). Although ALS-patients exhibited longer cognitive decision times than HCs overall, patients were not slowed on any of the individual tests in particular ($P = 0.158$). There were no differences in reaction times and errors between patients and controls on AVIP ($Ps > 0.233$).

Discussions and conclusions: Our study detected a specific deficit in the task requiring the comprehension of action sequencing in a group of non-demented ALS-patients. In contrast, no deficits were observed on action and object association tests as well as in audiovisual information processing. The deficits in action sequencing were confined to the accuracy of responses only and did not appear to influence cognitive decision times. Our findings suggest that action deficits observed in ALS patients might in fact be due to a sequencing deficit. Moreover, they raise the question whether sequencing deficits

are specific to language or possibly extend to other neuropsychological domains, constituting one of the basic cognitive deficits in ALS.

C48 PREFRONTAL LOBAR DETECTING: A MORE SENSITIVE BATTERY FOR SCREENING COGNITIVE IMPAIRMENT IN EARLY-PHASE ALS?

JI Y[1], WEI L[2], DENG M[1], WANG K[2], FAN D[1]

[1]*Peking University Third Hospital, Beijing, China,* [2]*Anhui Medical University First Hospital, Hefei, China*

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: prefrontal lobar dysfunction, prospective memory, early phase

Background: ALS is now considered as a multisystem disease with co-occurrence of frontotemporal syndrome. Imaging studies show the prefrontal lobe is the most involved area in ALS patients with behavioral and cognitive impairment.

Objective: The aim of this study is to find a screening battery to detect subtle cognitive deficits in ALS patients at early stage.

Methods: Eighty consecutive patients diagnosed as ALS according to the El Escorial criteria and 59 normal controls were matched for sex, age and education. Both groups were assessed with a series of neuropsychological tests state as follows: the Mini-Mental State Examination (MMSE), Neuropsychiatric Inventory (NPI), Frontal Behavior Inventory (FBI), verbal fluency test (VFT), Stroop Color Word Interference Test (CWT), and prospective memory (PM) including event-based prospective memory (EBPM) and time-based prospective memory (TBPM). To further explore the topic a subset of patients underwent further examination involving picture and music emotional perception, theory of mind or decision making (Iowa Gambling Task and risk-taking task).

Results: As previous studies reported, our patients also did not differ from normal controls on MMSE, but significantly differed on behavioral inventories including NPI (4.60 ± 4.78 *vs* 0.16 ± 0.59, $P<0.001$) and FBI (3.19 ± 3.59 *vs* 0.18 ± 0.54, $P<0.001$). However, we found our patients did not differ from normal controls in traditional neuropsychological tests on executive function including VFT and CWT. On the other hand, statistically significant differences were found between ALS patients and the normal controls on the prospective memory (EBPM: 5.55 ± 2.20 *vs* 6.37 ± 1.80, $P=0.043$; TBPM: 4.85 ± 1.51 *vs* 5.65 ± 1.10, $P<0.001$), faux pas task (23.13 ± 10.09 *vs* 28.91 ± 6.20, $P=0.002$) and the arousal of negative pictures (6.38 ± 1.32 *vs* 7.18 ± 1.12, $P=0.008$). More interestingly, we found TBPM is more sensitive than EBPM in early phase patients (possible and probable-laboratory supported ALS). We did not find differences between ALS patients and normal controls on the valence and recognition of pictures, music emotional perception, Iowa Gambling Task and risk-taking task.

Conclusions: Prefrontal lobar dysfunction does exist among ALS patients, and may be spread from medial to lateral part. Behavioral test, PM and theory of mind used in this study is more sensitive in detecting behavioral and cognitive impairment in ALS patients at early stage than the classical cognitive measures.

C49 DIAGNOSTIC VALIDITY OF THE ALS COGNITIVE BEHAVIORAL SCREEN

RUSH B[1], WOOLLEY SC[2], BOYLAN K[1]

[1]*Mayo Clinic, Jacksonville, FL, United States,* [2]*California Pacific Medical Center, San Francisco, CA, United States*

E-mail address for correspondence: rush.beth@mayo.edu

Keywords: cognitive screen, cognitive impairment, diagnostic validity

Background: Cognitive and behavioral symptoms of amyotrophic lateral sclerosis (ALS) are increasingly recognized. The ALS Cognitive Behavioral Screen (ALS-CBS) (1–2) is a 20 point screening measure with unclear diagnostic validity.

Objectives: To compare diagnostic classifications of cognitive impairment from the ALS-CBS to those from gold standard neuropsychological evaluation.

Methods: Retrospective review of 24 cases of ALS referred for evaluation of cognitive and behavioral symptoms between January 1, 2008 and March 31, 2010. Each participant was administered the ALS-CBS and a standard of care neuropsychological evaluation and received two diagnoses; one from the ALS-CBS and one from neuropsychological evaluation. The following diagnoses were considered: ALS, ALS with cognitive impairment/no dementia (ALS-ci), ALS with behavioral impairment/no dementia (ALS-bi), or ALS with frontal-temporal dementia (ALS-FTD). A score of ≤ 11 on the cognitive form of the ALS-CBS suggested cognitive impairment and a score < 5 suggested ALS-FTD. Diagnostic classifications from the ALS-CBS and the neuropsychological evaluation were compared and diagnostic validity was determined.

Results: Ten out of the 24 cases were female (42%), with a median age of 68 years (range: 43 - 83 years), median education of 14 years (range: 9 - 20 years), and median of 18 months (range: 6 - 55 months) duration between motor neuron disease symptom onset to cognitive evaluation. Fifty percent of cases had bulbar-onset disease. Neuropsychological evaluation confirmed cognitive impairment in 16 out of 24 cases (67% base rate; 9 ALS-FTD and 7 ALS-ci). There were 50% bulbar cases in the cognitively impaired and unimpaired groups. Using a cut-off score of ≤ 11 on the ALS-CBS, 46% cases (11/24) had cognitive impairment (8 ALS-FTD, 3 ALS-ci). This cut-off score yielded a sensitivity of 69% and a specificity of 100%. Of the 5 cases for which the ALS-CBS yielded a false negative diagnosis of cognitive impairment, 4 of the cases had a diagnosis of ALS-ci and 1 case had a diagnosis of ALS-FTD on neuropsychological evaluation.

Conclusion: The ALS-CBS demonstrates excellent specificity using a cut-off score of ≤ 11 for determining cognitive impairment. The ALS-CBS may have limited sensitivity to ALS-ci or cognitive impairment without dementia.

Discussion: Data support the validity of the ALS-CBS as a screen for cognitive impairment in ALS. The cut-off score of ≤ 11 on the cognitive form of the ALS-CBS is empirically justified particularly when ALS-FTD is present. Alternate cut-off scores or language screening items could improve the sensitivity of the ALS-CBS to ALS-ci when it is present.

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C50 LONGITUDINAL COGNITIVE SCREENING USING THE ALS COGNITIVE BEHAVIORAL SCREEN (ALS-CBS™)

WOOLLEY SC[1], MOORE DH[1], VALAN M[1], SPITALNY M[2], KUSHNER G[1], FORSHEW DA[1], MILLER R[1], KATZ J[1]

[1]*California Pacific Medical Center, San Francisco, CA, United States,* [2]*Research Computing, Mill Valley, CA, United States*

E-mail address for correspondence: Woolles@sutterhealth.org

Keywords: cognition, screening, longitudinal

Background: The ALS Cognitive Behavioral Screen (ALS-CBS™) has been validated as a clinical measure but its utility in research is unknown. Its ease of administration is ideal for longitudinal assessment of cognition in large cohorts.

Objectives: To evaluate cognitive screening data obtained from the WALS multi-center trial of lithium carbonate and to characterize cognitive status of this research cohort over time.

Methods: 109 patients with ALS were enrolled. The maximum cognitive score on the ALS-CBS™ is 20; scores at or below 10 are suggestive of frontotemporal dementia (1). The mean score for a clinical cohort (n=112) was 14.6 (1). Longitudinal cognitive change was calculated using the difference in scores over 12 months. Age of onset, duration of symptoms, forced vital capacity (FVC), ALSFRS-R scores, and fatigue were measured in the trial.

Results: The mean age of subjects was 56 years, mean ALSFRS-R at study initiation was 37.5, and mean FVC was 94.8%. Ninety-eight subjects completed the ALS-CBS™ longitudinally. The average cognitive score was 16.68 (2.67) at baseline and the cohort remained stable over 1 year (16.69). Only 7% of subjects revealed significant cognitive declines (>5 points) but 4% revealed significant increases. Declines in cognition were not associated with age, symptom duration, low FVC, low ALSFRS-R scores, fatigue, or drug side effects.

Discussion: The subjects in this clinical trial did not show significant decline over a 12-month period. This suggests that frontal lobe decline is not prominent among patients who meet inclusion criteria for a clinical trial. The mean cognitive score in the trial was higher than in a clinical cohort suggesting that patients enrolling in trials are less likely to be impaired than clinical patients. These results also differ from previous longitudinal research in a clinical cohort which revealed longitudinal cognitive decline (2).

Conclusion: Clinical trials may enroll patients with different cognitive profiles than those seen in routine clinic. This may relate to research patients needing to be motivated, having limited duration of disease due to inclusion criteria, or having higher FVC's which limits those enrolled with significant bulbar involvement. While the methodology does not allow us to generalize our conclusions, the lack of progression in this cohort also points towards a relative paucity of cognitive impairment in this group. The results add to increasing evidence that a screening tool is a practical way to monitor cognition and behavior longitudinally in large cohorts.

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C51 COMPUTERIZED FRONTAL ASSESSMENT OF AMYOTROPHIC LATERAL SCLEROSIS

POLETTI B, LAFRONZA A, SOLCA F, TICOZZI N, MORELLI C, MESSINA S, SILANI V

Department of Neurology and Laboratory of Neuroscience, 'Dino Ferrari' Center, University of Milan, IRCCS Istituto Auxologico Italiano, Milan, Italy

E-mail address for correspondence: poletti.barbara@gmail.com

Keywords: frontal functions, computerized tools, neuropsychological assessment

Background: The study of cognition and behaviour as a feature of Amyotrophic Lateral Sclerosis (ALS) is an evolving field still lacking a full consensus on terminology, diagnostic criteria, and clinical significance of any detected abnormalities. Marked discrepancies remain regarding the incidence of abnormalities and characteristics of the impairments that define ALS, frequently depending on the kind of cognitive tool used. Frontal alterations in ALS have been variously described and assessed in literature. Moreover, different degrees of frontal involvement in ALS starting from minimal frontal changes to overt Fronto-Temporal Dementia (FTD) have been documented, thus revealing the importance of detecting the whole spectrum of frontal involvement characterizing motor neurone disease's cognitive pattern. The terms ALSci (ALS with cognitive impairment), ALSbi (ALS with behavioural impairment), and ALS-FTD are developing concepts that aim to capture the key differences between the various clinical phenotypes. Computerized neuropsychological tools seem to be ideal in exploring and characterizing frontal cognitive functions.

Objectives: The purpose of this study was to analyze frontal cognitive functioning of ALS patients with frontal cognitive computerized measures.

Methods: Fifteen patients fullfilling El Escorial Criteria for ALS and fifteen controls underwent an extensive neuropsychological and psychodiagnostic assessment. Patients received the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRSr). Attentional skills and frontal functioning have been investigated with a computerized neuropsychological battery named TEA (Test of Everyday Attention). This battery, focused on attention and frontal functions, assess the ability of patients to selectively attend, sustain their attention, divide their attention between two tasks, switch attention from one task to another, and withhold (inhibit) verbal or motor responses. Clinical tools for assessing psychological and emotional status included: MOS 36-Item Short-Form Health Survey (SF-36), Beck Depression Inventory (BDI) and State-Trait Anxiety Inventory-Y (STAI-Y).

Results: Our data show quantitative differences in cognitive performances between patients and controls, with higher difficulties in the more complex attentional tasks for the former and better scores for the latter. Significant differences of data emerge on different frontal measures, while a general cognitive slowness characterized patient's performances. Patients displayed lower performances on frontal task of cognitive functioning. Subjects differed significantly for the presence of anxiety symptoms. Neuropsychological and psychological data were correlated with functional and respiratory parameters.

Discussion and conclusions: Computerized neuropsychological assessment seems an ideal tool in detecting small 'frontal' cognitive changes frequently observed in the cognitive frontal spectrum of ALS. Implications for clinical purposes will be discussed.

SESSION 8C CLINICAL TRIALS

C52 ESSENTIALS OF PHASE II CLINICAL TRIAL DESIGN

RAVINA B

Clinical Trials Coordination Center, University of Rochester, Rochester, NY, United States

E-mail address for correspondence: bernard.ravina@ctcc.rochester.edu

Keywords: phase II trials, trial design

Phase II clinical trials encompass a broad range of goals. These goals may include: assessment of safety, tolerability, logistics and feasibility; characterization pharmacokinetics and pharmacodynamics in the target population; refinement of the target population; evidence of mechanism of action ('target engagement'); prediction of efficacious doses, exposures, and responses; preliminary evidence of clinical efficacy or futility; characterization of selected biopharmaceutical and drug interaction and drug metabolism issues, as appropriate. Because of the broad range of goals, there is no single clinical trial design that is suitable for phase II. The design must be tailored to fit the goals of the study and the particular questions about the intervention. More than one study will generally be required to answer the key questions and lay the groundwork for phase III, comparative efficacy trials.

Phase II trials in neurodegenerative disorders pose unique challenges. These include problems with measuring target engagement and the pharmacodynamic activity of the intervention, and the relatively slow clinical evolution. These limitations may make phase II trials in neurodegenerative disorders longer and larger than desired or possible, leading to problems in the design and conduct of phase III trials. This is highlighted by the large number of negative phase III clinical trials and underscores the need for phase II clinical trials that are better able to predict subsequent success or failure in phase III.

This presentation will highlight critical questions in phase II using examples from other neurodegenerative disorders such as Parkinson's disease and Friedreich Ataxia: measurement of pharmacodynamic activity using biomarkers and strategies for dose selection. Additionally we will consider the predictive value of phase II designs. Certain phase II designs may be thought of as diagnostic tests that are meant to predict positive or negative results in phase III trials. In this framework, one should consider how the prior probability of success and the study design parameters, such as power and type I error rate, may affect the positive and negative predictive value of the phase II trial.

C53 THE 'SMALL' CLINICAL TRIAL: CHALLENGES IN DESIGN AND INTERPRETATION OF RESULTS

MCDERMOTT MP

University of Rochester, Rochester, NY, United States

E-mail address for correspondence: mikem@bst.rochester.edu

Keywords: phase II trial, sample size, research question

Clinical trials in rare diseases such as ALS pose many challenges given the limited available resources and numbers of willing trial participants. In this setting, feasibility constraints can lead to compromises in important principles of sound trial design. These principles include careful formulation of the research question, tailoring of the design to best answer the research question, and use of appropriate and efficient statistical methods for data analysis. Important design features include selection of an appropriate target population, choice of outcome measures, recruitment of an adequate number of trial participants, and use of methods to reduce or avoid bias (eg, randomization, blinding, and facilitating participant retention). The conduct of trials that are inadequately designed to address the research questions posed slows progress toward finding safe and effective treatments for ALS; this can be particularly problematic in middle development (Phase II). The lack of clear reporting and proper interpretation of trial results, especially for "small" trials with their inherent limitations, can likewise impede research progress. Properly designed Phase II trials and correct interpretation of their results can help avoid the inappropriate discarding of treatments from future consideration or inappropriate shift from equipoise in the research community in favor of a treatment.

This presentation will consider ways to address common difficulties encountered in the design and interpretation of clinical trials involving relatively small numbers of participants. These include precise formulation of a focused research question; carefully distinguishing between a 'negative' and an 'inconclusive' result concerning the potential efficacy (or activity) of a treatment; determining whether a seemingly 'positive' result concerning a treatment effect is to be believed; recognizing potential bias associated with the use of nonrandomized controls; and considering the impact of eligibility criteria on the generalizability of results. Examples from ALS and other disease areas will be used to illustrate the importance of formulating the right questions and addressing these questions in a rigorous manner to enhance decision-making in middle development.

C54 COMPARING ENDPOINTS: SLOPE OF ALSFRS-R HAS GREATER POWER THAN TIME TO LOSE SIX POINTS

MOORE DH, KATZ JS, MILLER RG, THE WALS STUDY GROUP

California Pacific Medical Center, San Francisco, CA, United States

E-mail address for correspondence: dmoore@cc.ucsf.edu

Keywords: ALSFRS-R, linear mixed effects model, primary endpoint

Background: A recent randomized trial of lithium used time to an event, in this case losing ≥6 points in ALSFRS-R score or death, as the primary endpoint. A log-rank test was used to judge whether lithium treatment was efficacious or futile.

Objectives: To compare the power of the time to an event endpoint with that based on estimating rate of decline (slope) of ALSFRS-R over time.

Methods: We used data from 246 untreated patients in a recent controlled trial with monthly ALSFRS-R to generate simulated data to compare the two endpoints. Simulated data was based on linear fits to observed data from real patients. Simulated trials assigned patients to two groups: treated and placebo. For the treated, the slope of ALSFRS-R decline over time was reduced; for the placebo it was unaltered. The

simulations recorded the numbers of patients with drops ≥6 points, or death, with slopes estimated in a linear mixed effects (lme) model fitted to the simulated data. For each simulation we recorded the P-value from the log-rank test with the P-value for testing a change in slope due to the treatment in the lme model.

Results: The results of the simulation clearly show that ability to detect a treatment effect is higher for the change in slope than for the time to event endpoint. For example, with 50 patients randomly assigned to each group (treatment and placebo) with up to 12 months followup, power was 0.67 for the slope change vs. 0.31 for the time to 6 point drop design when the simulated change in slope was a 40% reduction. When trial follow-up is shortened to 6 months, power was 0.85 for slope change vs. 0.15 for time to event.

Discussion: The time to an event endpoint is appealing because of its simplicity and lack of reliance on a specific model for rate of decline. However, power for time to event designs depend on the number of events so that patients that do not have drops ≥6 points contribute little to power. A slope analysis requires that rate of decline be reasonably linear but power increases with numbers enrolled. It appears that the advantage of estimating slopes over counting events is considerable when ALSFRS-R is the tool used to measure decline over time in ALS patients.

Conclusions: In ALS studies an endpoint based on slope comparisons is more powerful than one based on time to lose ≥6 points.

C55 ANALYSIS OF FUNCTIONAL SCORES DATA IN A STUDY OF DEXPRAMIPEXOLE FOR AMYOTROPHIC LATERAL SCLEROSIS IN THE CONTEXT OF NON-TRIVIAL DISCONTINUATIONS AND DEATHS

INGERSOLL E[1], ARCHIBALD D[1], SCHOENFELD D[2], MOORE DH[3], GRIBKOFF V[1], MATHER J[1], CUDKOWICZ M[4], SHEFNER J[5], MILLER RG[3], BOZIK M[1]

[1]*Knopp Neurosciences Inc., Pittsburgh, PA, United States,* [2]*Harvard School of Public Health, Boston, MA, United States,* [3]*California Pacific Medical Center, San Francisco, CA, United States,* [4]*Massachusetts General Hospital, Boston, MA, United States,* [5]*SUNY Upstate Medical University, Syracuse, NY, United States*

E-mail address for correspondence: eingersoll@knoppneurosciences.com

Keywords: dexpramipexole, clinical trial, ALSFRS-R

Background: Diseases of progressive deterioration like ALS present unusual challenges in analyzing clinical outcomes in interventional trials. The typical method, modeling slopes of functional change using a linear mixed effects model (LMEM), is conceptually valid, but fails to distinguish deaths from other discontinuations and does not reflect death as a worse outcome than survival with any degree of functional decline. Furthermore the LMEM assumes linearity of decline over time and that discontinuations and deaths are missing at random. An intent-to-treat analysis of mortality requires post-study assessments of vital status for subjects who discontinue.

Objective: To evaluate the clinical effects of dexpramipexole ([6R]-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine; KNS-760704) in a recent ALS study (KNS-760704-CL201; 'CL201') (1) in the context of imbalances in the rates of discontinuations and death between treatment groups by using multiple analytical methods.

Methods: In Part 2 of CL201, subjects were randomized (double-blind) to receive dexpramipexole 50 mg (n=48) or 300 mg (n=44) for 24 weeks. Clinical status was, in part, measured by ALSFRS-R and survival. Data were analyzed using 1) LMEM for slopes, 2) LMEM with imputation of zero scores for the first post-death visit of subjects who died ('zero imputation'), 3) Kaplan-Meier life table estimates, and 4) analysis of subject rankings (2) on mortality and change from baseline in ALSFRS-R.

Results: Twenty-one subjects discontinued (14 in the 50 mg group and 7 in the 300 mg group), including 12 deaths (9 in 50 mg and 3 in 300 mg, P = 0.071). Three of the 12 deaths were in subjects who had previously discontinued for other reasons (2 in 50 mg and 1 in 300 mg). The unadjusted mean slope in Part 2 of CL201 for subjects who died was steeper than for survivors (-1.80 vs. 1.08, respectively). The same pattern was observed for all discontinuers vs. completers. The LMEM analysis showed a 20% difference in slopes favoring 300 mg (P = 0.178), which increased to 43% with zero imputation (P = 0.018). The joint rank test of mortality and ALSFRS R also favored the 300 mg group (P = 0.046).

Discussion and conclusions: In ALS trials, discontinuations and deaths complicate the interpretation of observed cases. Slope estimates per se do not reflect the seriousness of death. Zero imputation is useful only to illustrate effects of bias as a sensitivity analysis. A true intent-to-treat analysis of mortality requires post-study follow-up for all subjects who discontinue. The joint-rank test is based on an intuitively reasonable ranking of outcomes with appropriate weighting of death outcomes, which involves minimal statistical assumptions. This study demonstrates that the joint-rank test in ALS is a novel, powerful method that bears further investigation as a primary analysis method in future trials.

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SESSION 9A AXONAL FUNCTION AND PATHOLOGY

C56 AXON DEGENERATION MECHANISMS: NEW INSIGHTS FOR SELECTIVE VULNERABILITY OF MOTOR UNITS IN AGEING AND DISEASE

COLEMAN MP, MILDE S, CONFORTI L, GILLEY J

The Babraham Institute, Cambridge, United Kingdom

E-mail address for correspondence: michael.coleman@bbsrc.ac.uk

Keywords: Wallerian degeneration, dying back, axonal transport

Early loss of axons and synapses and axonal transport impairment are common to many neurodegenerative diseases including motor neuron disease but the molecular events that may link them are poorly understood. Axonal transport defects or axonal spheroids occur in amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, Parkinsonism, multiple sclerosis, traumatic brain injury and glaucoma. Axonopathy in hereditary spastic paraplegia, peripheral neuropathy and motor neuron disease sometimes reflects loss or damage to the transport machinery itself and the substantial decline in transport during normal ageing could contribute to age-related 'dying back' disorders.

Intriguingly, a general disruption of anterograde delivery can lead to a specific axon degeneration pathway. Axon transection abolishes transport from the soma but addition of a single protein is sufficient to delay the ensuing Wallerian degeneration by tenfold. This 'slow Wallerian degeneration' protein (WldS) is an aberrant protein that arose in a healthy mutant mouse and combines sequences from nicotinamide mononucleotide adenylyltransferase Nmnat1 and ubiquitin ligase Ube4b. Mislocalisation or overexpression of Nmnat1 or Nmnat3 have similar protective effects.

In contrast, depletion of an endogenous Nmnat, the labile isoform Nmnat2, causes Wallerian-like degeneration of uninjured neurites in primary culture. WldS prevents this degeneration, probably by compensating for loss of Nmnat2 in an enzyme-dependent manner. However, the Nmnat function that promotes axon survival may not be NAD+ synthesis. Nmnat2 is abundant in the Golgi apparatus but live imaging shows it is also rapidly and bidirectionally transported along neurites. Our data suggest it is targeted to vesicular structures by palmitoylation, where it frequently comigrates with other Golgi-derived proteins but not with mitochondria.

In healthy axons, rapid and constant delivery of this essential protein seems to balance its degradation by the ubiquitin proteasome system, maintaining its level above the threshold needed for axon survival. When axonal transport or neuronal metabolism fails, we propose that failure to replace natural turnover of Nmnat2 limits axon survival. Deficiencies in other axonal cargoes take far longer to limit survival when loss of Nmnat2 is, indicating an important therapeutic window for some disorders if this pathway were appropriately targeted. It is essential now to extend these data *in vivo* and to understand the upstream and downstream events.

C57 CELLULAR TRANSPORT DYSFUNCTION IN SOD1G93A TRANSGENIC MICE AND NSC34 CELLS

FARG M[1], TURNER B[2], WALKER A[1], HORNE M[2], ATKIN J[1,2]

[1]*Department of Biochemistry, La Trobe University, Melbourne, Australia,* [2]*Howard Florey Institute, University of Melbourne, Melbourne, Australia*

E-mail address for correspondence: m.farg@latrobe.edu.au

Keywords: ER-Golgi, dynein, ER stress

Background: Motor neurons are large cells with long axons that have high synthetic and energetic requirements, placing a heavy demand on cellular transport processes. The motor protein dynein/ dynactin is involved in both axonal transport and transport of proteins from the endoplasmic reticulum (ER) to Golgi apparatus. Impaired cellular and axonal transport and disruption to dynein and dynactin function are linked to motor neuron degeneration in ALS. Dynein also physically interacts with mutant but not wildtype SOD1 and it co-localises with mutant SOD1 inclusions. Furthermore, impairment of dynein-dynactin function results in Golgi fragmentation. which occurs in both sporadic and SOD1-mediated familial human ALS and in transgenic $SOD1^{G93A}$ animals. Recently there has been a surge of publications describing the importance of ER stress in ALS. We described induction of the whole unfolded protein response in both transgenic $SOD1^{G93A}$ animals and in sporadic ALS patients. ER stress is triggered either by the presence of misfolded proteins in the ER or by inhibition of general protein transport from the ER.

Objectives: We hypothesised that disruption in ER- Golgi trafficking triggers ER stress in ALS. Anterograde and retrograde transport proteins were examined in transgenic $SOD1^{G93A}$ mice and in NSC34 cell lines transfected with mutant and wildtype SOD1. We examined dynein, Rab1 and the coat protein complex II (COPII), which transports proteins from the ER to the Golgi apparatus in coated vesicles.

Methods: NSC34 cells transfected with SOD1 construct were immunostained with dynein intermediate chain, COPII and GM130 antibodies and confocal imaging was performed. Lysates from either NSC-34 cells or transgenic mice $SOD1^{G93A}$ were coimmunoprecepitated with COPII,dynein and Rab1, followed by Western blotting with SOD1.

Results and conclusions: A physical interaction between mutant (but not wildtype SOD1) with both COPII and the dynein intermediate chain was detected in transgenic $SOD1^{G93A}$ mice. This was detected in pre-symptomatic animals as early as postnatal age 10 days, which is 20 days prior to the onset of ER stress. This interaction was confirmed in NSC34 cell lines expressing SOD1 mutants A4V, G85R and G37R. COPII also co-localises with mutant SOD1 inclusions in NSC-34 cells. We also showed that secretion of brain derived neurotrophic factor (BDNF) was reduced in NSC-34 cell lines expressing mutant SOD1, thus implying dysfunction of the ER-Golgi secretory pathway in ALS. Mutant SOD1 also bound aberrantly to other proteins involved in ER to Golgi trafficking including Rab1, which is

involved in the docking of vesicles between the ER and Golgi apparatus. These data suggest that impairment in cellular trafficking between the ER-Golgi apparatus occurs as an early event in ALS. These events occur prior to ER stress, suggesting that cellular trafficking dysfunction triggers ER stress in ALS.

C58 *IN VITRO* MODEL SYSTEMS FOR NMJ FORMATION UTILIZING MOTONEURONS FROM ADULT RAT, MOUSE AND SOD1 TRANSGENIC MOUSE

DAS M[1,2], BHARGAVA N[1], STANCESCU M[1], RUMSEY J[1,3], HICKMAN J[1]

[1]*University of Central Florida, Orlando, FL, United States,* [2]*Indian Institute of Technology, Kanpur, India,* [3]*Sanford-Burnham Medical Research Institute, Lake Nona, FL, United States*

E-mail address for correspondence: jhickman@mail.ucf.edu

Keywords: in vitro, adult motoneuron, NMJ

Background: *In vitro* model systems for research in ALS and MND suffer from the limitation that they are composed of embryonic cells for the most part and there are few examples of neuromuscular junction (NMJ) formation in these culture systems. Model systems composed of adult motoneurons from rat, mouse, and SOD1 mouse, that exhibit neuromuscular junctions on the myotubes derived from adult satellite cells, would be a great benefit to the field. These systems could form the basis for basic research model systems as well as for high-content screens for drug discovery.

Objectives: To engineer defined, serum-free culture systems utilizing tissue or motoneurons from adult rat, mouse and SOD1 transgenic mice and demonstrate NMJ formation on myotubes derived from satellite cells from adult muscle. To then use these *in vitro* systems to study NMJ formation between diseased and healthy tissue or in the presence of factors known to cause ALS or MND type deficits.

Methods: Cultures were evaluated utilizing morphological analysis, immunocytochemical evaluation for phenotype and electrophysiology to determine electrical properties of the regenerated motoneurons as well as myotube function. NMJ formation was established by immunocytochemical and videography. All cell types, as well as co-cultures, were done in a defined, serum-free culture system utilizing a non-biological, silane based culture surface.

Results: Regeneration and recovery of electrical properties was achieved for adult rat motoneurons (1), adult mouse (2) and SOD1 transgenic mice. Single temporal doses of the neurotransmitters serotonin, glutamate (N-acetyl-DL-glutamic acid) and acetylcholine-chloride lead to the full electrophysiological functional recovery of approximately 60% of the adult spinal cord neurons. NMJ formation has also been clearly demonstrated in this defined, serum-free, *in vitro* system. The clustering of AChRs using alpha-bungarotoxin and their co-localization with synaptophysin vesicles was analyzed immunocytochemically and the appearance of striations after this time indicated that the co-cultures contained about twice the number of myotubes showing striations. Cultures survived for over 2 months with viable cellular properties.

Discussion and conclusions: The ability to routinely culture long-term the adult motoneurons of rat, mouse and SOD1 transgenic mouse opens new avenues to investigate *in vitro* NMJ formation. The ability to now culture the neurons from SOD1 mice after they begin expressing behavioral deficits could provide a new experimental tool to investigate this disease. This improved system supports the goal of creating a physiologically relevant tissue engineered motoneuron plus skeletal muscle construct to develop functional bio-hybrid devices to analyze NMJ activity. Furthermore, we believe this serum-free culture system will be a powerful tool in developing advanced strategies for regenerative medicine in ALS and MND.

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C59 INCREASED RATE OF MATURATION OF ELECTRICAL PROPERTIES OF SOD1 MOTONEURONS

QUINLAN K, SCHUSTER J, FU R, SIDDIQUE T, HECKMAN CJ

Northwestern University, Feinberg School of Medicine, Chicago, IL, United States

E-mail address for correspondence: katharina-quinlan@northwestern.edu

Keywords: SOD1 mouse, electrophysiology, postnatal development

Background: Spinal motoneurons are highly vulnerable to ALS, rapidly degenerating while other neurons remain healthy. Previous research using standard animal models, mutant SOD1 mice, has revealed many deficits in cellular properties at different time points in the disease, starting in the early postnatal stages. However which properties in the motoneurons are the first to become abnormal has not been systematically investigated.

Objectives: Therefore we undertook a more comprehensive approach comparing the development of multiple markers excitability during the first 12 postnatal days (P0-12) in $SOD1^{G93A}$ mice. We tracked more than 25 intrinsic properties associated with excitability in $SOD1^{G93A}$ motoneurons to compare their postnatal development to non-transgenic and $SOD1^{WT}$ motoneurons.

Methods: Using whole cell patch clamp of over a hundred motoneurons in acute slices from the lumbar spinal cord of mice P0 to P12, we tracked the developmental changes relating to excitability.

Results: We show changes in several properties with both age and $SOD1^{G93A}$ mutation, including increases in both $Na+$ and $Ca^{2}+$ components of the persistent inward current, increased input conductance, decreased action potential duration including faster rates of rise and fall, and faster decay of the afterspike after hyperpolarization. Though there were a few exceptions (current at which firing onset and offset occur, I_{ON} and I_{OFF}, and hyperpolarization of resting membrane potential), overall the changes in $SOD1^{G93A}$ motoneurons were consistent with those observed during postnatal development. To test this, changes due to mutation were normalized and plotted compared to the normalized changes due to maturation. Results were significant ($P < 0.0001$), indicating that $SOD1^{G93A}$ motoneurons are undergoing the same changes

observed during postnatal development, except the SOD1 motoneurons seem to be progressing more rapidly towards the mature state.

Discussion and Conclusions: Overall, the differences in the $SOD1^{G93A}$ motoneurons closely fit a profile of an increased rate of postnatal maturation. The resulting early achievement of the adult state in these motoneurons may trigger an overall accelerated rate of aging in motoneurons and thus increase motoneuron vulnerability.

C60 ASSESSING MOTOR NEURON LOSS IN THE ALS RAT WITH ELECTRICAL IMPEDANCE MYOGRAPHY

WANG L, LI J, RUTKOVE S

Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, MA, United States

E-mail address for correspondence: srutkove@bidmc.harvard.edu

Keywords: MUNE, impedance, rat

Objective: To evaluate the relationship between electrical impedance myography (EIM) and standard electrophysiological measures, including motor unit number estimation (MUNE), and survival in the SOD1 G93A amyotrophic lateral sclerosis (ALS) rat.

Background: Current electrophysiological methods for quantifying motor neuron loss in ALS in both pre-clinical and clinical studies are limited. The most commonly used method, MUNE, is relatively unreliable early in the disease course, is time-consuming to perform, and can only be applied to a limited set of muscles. Past and ongoing studies in human subjects suggest that EIM may be a powerful indicator of disease progression. In addition to providing a sensitive measure of motor neuron loss, EIM is much easier to perform, may provide reliable data early in the disease course, and is readily applied to most muscles. How well EIM correlates to MUNE, identifies disease onset, and predicts survival, however, remains unknown.

Methods: Sixteen male ALS rats underwent repeated MUNE, EIM, weight and behavioral assessments starting from approximately 85 days of age. MUNE was performed using an incremental technique stimulating the sciatic nerve and recording from the entire distal leg. EIM was performed over the gastrocnemius muscle. Several different EIM parameters were evaluated. Animals were sacrificed when they became unable to right themselves after 15 seconds after being laid supine.

Results: EIM parameters effectively tracked disease progression in the animals and mirrored the MUNE. EIM's 50 kHz phase correlated strongly with MUNE, with $r = 0.88$ ($P < 0.001$). EIM 50 kHz phase was also a sensitive indicator of disease onset, showing reductions by 98 days, whereas MUNE and weight assessments did not demonstrate alterations until greater than 110 days. Additionally, the rate of decline in EIM 50 kHz phase from the time of disease onset until 140 days of age correlated strongly with the time of death several weeks later ($r = 0.75$, $P<0.01$) whereas MUNE obtained over the same time period did not.

Discussion: These results showed that EIM data strongly correlate with MUNE, supporting its essential nature as an indicator of motor neuron loss. However, EIM parameters also identified disease onset and predicted time of death earlier and with more accuracy than MUNE, supporting its clinical relevancy.

Conclusions: Since EIM can be applied to virtually any muscle or muscle group, is painless, and is rapid to perform with only minimal training, it deserves to play an important role in assessing drug efficacy in future clinical therapeutic studies in ALS.

C61 EVIDENCE OF EARLY SOMATO-DENDRITIC ALTERATIONS IN LUMBAR MOTONEURONS OF SOD1 JUVENILE MICE

FILIPCHUK A[1,2], PAMBO-PAMBO A[1], LIABEUF S[1], BROCARD C[1], KULAGINA I[2], KOROGOD S[2], GUERITAUD JP[1], DURAND J[1]

[1]*CNRS UMR6196 and Université de la Méditerranée, Laboratoire de Plasticité et Physiopathologie de la Motricité, Marseille, France,* [2]*Dnipropetrovsk National University, Dnipropetrovsk, Ukraine*

E-mail address for correspondence: durand@dpm.cnrs-mrs.fr

Keywords: morphology, modeling, development

Background: Recently we showed that lumbar motoneurons from superoxide dismutase 1 (SOD1) mutant mice (Amyotrophic Lateral Sclerosis, (ALS) mouse model) are affected in their electrical properties and have over-branched dendrites at an early postnatal age (P8-P10) (1–3). In this work, we addressed the questions of whether the abnormal branching is already present at birth and whether it might be due to changes in the synaptic organization to motor nuclei.

Objectives: To understand how and why SOD1 neonate motoneurons grow abnormally.

Methods: We identified and recorded lumbar motoneurons in wild type (WT) and in SOD1 juvenile mice at postnatal days P3-P10 using intracellular recording and staining procedures as previously described (1). The labeled motoneurons were then reconstructed using neurolucida system and morphometric and topologic parameters were analyzed. We used computer models of 3D reconstructed WT and SOD1 motoneurons to assess the functional consequences of the morphological changes (4). We also used specific antibodies directed against synaptophysin and GFAP and immunohistochemistry to study presynaptic terminals and glial processes in motoneuronal pools from ventral spinal cords.

Results: These experiments revealed that WT and SOD1 motoneurons have a comparable number of dendritic branches at P3-P4 while certain morphological parameters are already different at that early age. The number of branches in WT motoneurons did not increase between P3 and P9 indicating that motoneuron grows by elongation instead of developing new branches at that period. On the contrary, SOD1 motoneurons over-branched precisely between P4 and P8. The mean number of branches and the mean total dendritic length are significantly higher in SOD1 motoneurons compared to those measured in non transgenic WT motoneurons at P8-P9. The abnormal branching occurs in both SOD1 G85R and G93A mutant mice. Immunofluorescent labeling of synaptic terminal reveals significantly lower values at P3 in SOD1 motoneuronal pools compared with age-matched WT pools. Conversely, glial staining was lower in the ventral and lateral part of the spinal cord from SOD1 mice at P8 compared with

age-matched WT mice. Some glial tissue might be replaced by supernumerary dendrites.

Discussion and conclusions: Our results show that lumbar motoneurons of SOD1 mice have an abnormal branching with an onset between P4 and P8 and suggest that this abnormal branching might be due to lower number of presynaptic terminals reaching the motoneuronal pools. These morphological changes might be among the earliest compensatory mechanisms detected in SOD1 motoneurons.

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SESSION 9B INTERNATIONAL PERSPECTIVES ON CARE PRACTICE

C62 PALLIATIVE CARE – ACCESS TO SERVICES AND SUPPORT FOR PROVIDERS – A PROJECT WITH OUTCOMES

HARRIS R

MND Association of Victoria, Melbourne, Victoria, Australia

E-mail address for correspondence: rharris@mnd.asn.au

Keywords: palliative care, access, key worker

Background: People with ALS/MND were underrepresented in palliative care services in Victoria. Palliative care providers were reluctant to accept referrals of people with ALS/MND. The role and responsibility of palliative care providers appeared confused, and people with ALS/MND were reluctant to access services that could improve their quality of life.

Objectives: To establish a framework that triggers referral to a palliative care services; develop and describe mechanisms that support communication; articulate the roles of the palliative care provider, and identify gaps in current palliative service provision for people with ALS/MND and make recommendations for resolving them.

Methods: Data collection was based on a literature review and discussion with people living with ALS/MND, past and present carers, palliative care service representatives, and key opinion leaders within the palliative care sector. Topic specific information from the literature review was combined with interview themes to construct a series of recommendations for the coordinated integration of palliative care into the overall management of people living with ALS/MND.

Results: The project found that there was not an existing framework that could be implemented to integrate palliative care into the overall care received. Palliative care workers did not feel confident in their knowledge of ALS/MND, and people with ALS/MND had a distorted understanding of palliative care and the services it could offer. Palliative care workers and clients with ALS/MND reported that rarity and rapid progression of the disease caused problems with coordinating care between numerous agencies. Inpatient palliative care reported higher resource inputs were needed. This was supported by the literature showing other patients receive less care when a client with ALS/MND was admitted because time and resources were redirected. The lack of appropriate respite accessible in a timely manner was highlighted by carers as a significant unmet need, causing considerable emotional, psychological and physical demands. The presence of after hours palliative care support while not widely used offered carers and clients peace of mind as the disease progressed.

Discussion and conclusions: The research highlighted the fear of palliative care staff in working with ALS/MND of which they had little knowledge or understanding. It further highlighted the misapprehension that once a person was admitted to palliative care that the palliative care agency had responsibility for all of the clients needs. The report recommended the development of a key worker model within palliative care services to promote early referral, support and deliver education and coordinate between service providers. A comprehensive education and support program was to be developed. Guidelines and a mechanism for supplementary funding to address identified high care and ongoing needs need to be developed. The respite needs and after hours palliative care support be the subject of policy considerations.

C63 WWW.ALSHOME.DE: WEB-BASED SELF-ASSESSMENT OF SYMPTOMS IN ALS

MAIER A, LINKE P, HOLM T, DULLINGER J, MÜNCH C, BORISOW N, BAHRKE R, JANZEN C, MEYER T

Charité, Department of Neurology, Berlin, Germany

E-mail address for correspondence: andre.maier@charite.de

Keywords: web-based self-assessment, self-management, outpatient care

Background: Self-assessment of symptom progression and quality of life in chronic diseases is of increasing importance in clinical research and specialised outpatient healthcare. Against this background, we developed the Internet portal www.ALShome.de which provides online access to the ALS Functional Rating Scale, revised (ALSFRSr) and other established self-assessment questionnaires. In home care, an internet-based self-assessment is a possible perspective for the monitoring of ALS-associated symptoms.

Objective: To survey web-based self-assessment of ALS symptoms using the ALS Functional Rating Scale, revised (ALSFRSr) and other established self-assessment questionnaires.

Method: www.ALShome.de was created as a secure and closed Internet portal for patients. The application was developed in c-sharp (c#) with the persistent data storage being realized via an MS-SQL database throughout. Data are captured by generic questionnaires, are visualised on the website and administrated via a content management system. Patients are assigned a discretionary number of online visits. In a prospective, controlled and stratified study, patients conduct a self-assessment of ALS-associated symptoms. The study protocol designates 300 patients to visit the website weekly over a 52-week period.

Results: We have already included 62 patients (25 female, 37 male) in this study, 93.5 % (n = 58) of whom still perform weekly visits to the website filling in the ALSFRSr questionnaire nine weeks on. Reasons for discontinuation were the psychological strain perceived by patients when being confronted with the later stages of the disease (n = 2) and an excessive time burden caused by analogue dial-up Internet connections at a speed of less than 56 kbytes/s (n = 2).

Discussion: The web-based self-assessment of ALS symptoms in a home care environment complements the well-established application of the ALSFRSr in outpatient departments. The current study was able to demonstrate the medical, logistical and technical feasibility. The low drop out rate expresses the high acceptance of this online self-assessment tool among ALS patients. The study supports the hypothesis that innovative elements of self-management perspectively gain significant relevance in outpatient care.

Acknowledgements: The study is supported by the Air Berlin fund for ALS therapy research at the Charité.

C64 VOICE BANKING TO FACILITATE COMPLIANCE WITH SPEECH GENERATING AUGMENTATIVE COMMUNICATION

SINGLETON E[2,3], ROSENFELD J[1]

[1]*UCSF-Fresno Neurology, Fresno, California, United States,* [2]*Neuroscience Outpatient Rehabilitation Center, Fresno, California, United States,* [3]*Central California Neuroscience Institute, Fresno, California, United States*

E-mail address for correspondence: jrosenfeld@fresno.ucsf.edu

Keywords: voice-banking, natural voice, AAC-compliance

Background: Patients living with ALS/MND often experience a partial or total loss of speech and may require the use of a speech-generating device (SGD) to communicate. Despite the need and significance of augmemtative and alternative communication (AAC), compliance is variable and issues contributing to optimal compliance are not well understood. Selection of the designated voice used with a SGD may directly affect compliance.
Voice banking uses a patient's own pre-recorded, natural voice and may increase motivation and compliance for use of their SGD. Voice banking requires planning as recordings are made prior to loss of functional speech. Pre-recorded messages can then be programmed onto most available SGDs.
We will be presenting the organization and phases of our voice banking program and efforts to determine the role of voice banking in enhancing AAC compliance.

Objectives: 1) To introduce the voice banking program at the UCSF-Fresno, Central California Neuroscience Institute; 2) To determine the role of voice banking in enhancing compliance with the use of speech generating AAC devices.

Methods: Phase 1 of our program is focused on exploring issues that both enhance and impede compliance of patients who are currently using speech generating devices. Selective emphasis is placed on the role of the SGD voice. The patient's satisfaction with that voice and its impact on compliance are evaluated by written questionnaire administered to both the patient and the caregiver. Phase 2 involves assessment of voice banking on improving AAC compliance. Compliance and satisfaction are initially measured in patients receiving speech generating AAC devices utilizing commercially available voices. This population of patients will also have pre-recorded (banked) their natural voice prior to onset of their dysarthria. The banked voice will then be replaced in their SGD and the patient's compliance and satisfaction are reassessed serially.

Results: We will present the results of our phase 1 and early phase 2 data from this ongoing longitudinal project. Data highlighting issues impacting compliance in our population will be presented. Methodology and early results on the logistics of voice banking in phase 2 will also be presented.

Conclusion: Our research program in voice banking at UCSF-Fresno, Central California Neuroscience Institute is an ongoing, multi-phase program designed to determine the role of voice banking in enhancing compliance with AAC. This presentation is the first phase of this novel ongoing initiative. The results of this program will identify key areas impacting compliance and the logistics of implementing a voice banking program at other institutions.

C65 AMYOTROPHIC LATERAL SCLEROSIS PATIENTS' SELF-REPORTED SATISFACTION WITH ASSISTIVE TECHNOLOGY

GRUIS K[1], WREN P[2], HUGGINS J[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Oakland University, Rochester, MI, United States*

E-mail address for correspondence: kgruis@umich.edu

Keywords: rehabilitation, survey, assistive technology

Background: Clinical management recommendations for amyotrophic lateral sclerosis (ALS) patients with physical impairments include medical provider assessment and prescription of assistive devices to improve patients' function, maintain independence, and decrease fatigue. However, there is little information about ALS patient reported usefulness of, and satisfaction with commonly prescribed assistive devices.

Objective: We assessed ALS patients' self-reported satisfaction with thirty-three assistive devices using a telephone survey.

Methods: Ninety-six ALS patients were identified from our multidisciplinary clinic, and 63 were available via phone. A structured, telephone survey of those patients available by phone was conducted to assess demographics, ALS functional rating scale-revised (ALS-FRS-R), and assistive technology. Assistive technology frequency of use, perceived usefulness, and satisfaction with 33 devices was performed. Frequency of use was recorded on a scale of 1 - 5 (1=never, 2=rarely, 3=sometimes, 4=often, and 5=always) and designated as 'high use' if scored as often or always by ≥ 20% respondents. Usefulness and satisfaction were recorded on a 1 to 10 scale where 1, 2=very low; 3, 4=low; 5, 6=medium; 7, 8=high; and 9, 10=very high. Frequencies and percentages or medians and interquartile ranges (IQR) were calculated.

Results: Survey respondents median age was 62 years (52, 72); 37 (59%) were male and 52 (83%) reported limb-onset symptoms. The median duration between the diagnosis and survey was 26 months (17, 50), and the median ALS-FRS-R score was 25 (18, 33). Sixteen (48%) of the devices were designated high use. Of these, slip-on shoes and all bathroom devices received a very high rating for both usefulness and satisfaction; ankle braces and transfer boards were rated very high for usefulness with high satisfaction; speaker phones and electronic seating controls were rated high for both usefulness and satisfaction. Although walkers, motorized wheelchairs, personal digital assistants, and laptop computers were also high use and were given a high usefulness rating, they only received a medium satisfaction rating. The button hook and dressing stick received low ratings, and the long-handled reaching tool received very low ratings for both usefulness and satisfaction, despite being designated high use. Electronic speaking devices were used by only eight (13%) of ALS respondents, while writing on paper was used by forty-three (68%). Interestingly, both of these communication devices received the same, medium, rating for both usefulness and satisfaction.

Conclusions: This cross-sectional telephone survey of a cohort of ALS patients found that bathroom adaptive devices were uniformly the most frequently used and highly rated assistive devices for both how well the devices worked and satisfaction. Additionally, of those assistive technologies used often or always by ≥ 20% of respondents, the ankle brace, transfer board, slip-on shoes, speaker phone, and electronic seating controls were ranked highly for both usefulness and satisfaction.

C66 "THE DOCTOR SAID MND WOULDN'T AFFECT MY SEXUALITY … BUT IT HAS"

TAYLOR B

Oxford Brookes University, Oxford, United Kingdom

E-mail address for correspondence: bmtaylor@brookes.ac.uk

Keywords: sexuality, sexual expression, relationships

Background: Sexuality is a holistic concept that is individual and integral to each person throughout his or her lifetime (1). It influences how we perceive ourselves and is expressed in a variety of ways, including the way we dress, through touch, roles and relationships (1,2). Apart from studies on sexual function (3), little is known about the impact of MND on sexuality.

Objectives: This qualitative study explores the lived experiences of patients and partners of patients living with MND in the UK.

Method: One to one interviews were conducted with 13 patients and 10 partners of patients at different stages along the disease trajectory. Each person was interviewed twice and couples were interviewed separately. It was not a requirement of the study that people were in a partnered relationship.

Results: MND does have an impact upon peoples' sexuality. It causes changes in peoples' physical appearance and affects their self-concept and self-esteem. Participants have described how MND affects their self-confidence, and impacts upon their intimate and sexual relationships in a variety of ways, and also raises concerns about fertility. Health care professionals rarely speak about these issues, leaving individuals to find their own ways of managing change.

Discussion: Sexuality is an important aspect of peoples' lives that means different things to different people. When MND affects peoples' sexuality, some people are able to adapt and change, whilst others experience significant loss.

Conclusion: There is more to sexuality than erectile function. Telling people that their sexuality will not be affected does not recognise the psychosocial effects of MND or the practical and emotional interplay that is involved in sexual relationships. Recommendations are made for how this could be managed in healthcare practice.

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C67 THE NATION'S FIRST ALS GREEN HOUSE® NURSING HOME RESIDENCE: A TECHNOLOGICAL APPROACH TO CARE

BERMAN B, KRAKOW M, SALING S

Chelsea Jewish Foundation, Chelsea, MA, United States

E-mail address for correspondence: barry0630@aol.com

Keywords: technology, nursing care

Background: The Leonard Florence Center for Living (LFCL) in Chelsea Massachusetts opened the first-in-the-nation ALS Residence in February 2010. The ALS Residence is part of the LFCL Green House® nursing home, a model which deinstitutionalizes care while providing skilled nursing care in a home environment. In the ALS Residence, ten residents have their own private bedrooms and bathrooms, and share a common living, dining and kitchen area. Universal care workers and clinical staff provide care and services on an as needed basis, and there are no institutional trappings. This fully automated house uses state-of-the-art technology to allow wheelchair-dependent individuals with little or no mobility to live their lives fully – open and close shades and doors, control lighting and heat/air conditioning, control audio-visual components, and communicate with others, all possible with hand or eye movement technology. The ALS Residence has a full ventilator program, available when needed.

Objectives: 1) Develop an ALS Residence to provide care in a home environment. 2) Develop technology that will be integrated throughout the building, allowing residents to maintain and/or regain independence. 3) Provide care to ten residents at a time, refining nuances of care in the first ALS Residence. 4) Develop a model that can be replicated in other areas of the country/world.

Methods: A landscape architect who has ALS worked with architects, contractors and engineers on all design elements of the building to create the most technological advanced ALS Residence possible, with the goal of providing independence and mobility to those living with ALS. This same individual consulted with the clinical care staff on all aspects of providing clinical care. The ALS Association Massachusetts Chapter provided ongoing consultation and input into the project.

Results: The first residents moved into the ALS Residence in late February 2010. Qualitative information indicates that residents are seizing control of their lives. For the first time in years, residents are able to open doors for themselves, turn lights on and off, and control the environment in their bedrooms and homes. This mobility and independence is promoting interaction among those living in the ALS Residence as well as among the ALS patients and those within the LFCL. We have received inquiries from all over the country requesting information about and/or admission into the ALS Residence.

Discussion and conclusions: The ALS Residence provides residents with unprecedented mobility, independence and dignity, providing a technological cure for ALS while we wait for the medical cure. This model can be replicated in other areas of the country, providing similar independence to others. Consultation with local clinics serving ALS patients as well as with the local and national chapters of the ALS Association will continue as we plan for project replication.

SESSION 9C CLINICAL TRIALS

C68 MORE POWER TO YOU: IMPACT OF TRIAL DESIGN ON ADHERENCE AND OUTCOME

CUDKOWICZ M

Massachusetts General Hospital Harvard Medical School, Boston, MA, United States

E-mail address for correspondence: mcudkowicz@partners.org

Keywords: trial conduct, compliance, retention

Study conduct can greatly impact interpretability of trial results and the efficiency of developing new treatments for ALS. Study conduct includes adherence to the treatment intervention (medications, exercise, etc), study visits and study procedures. Using data from published clinical trials and trials conducting by the Northeast ALS consortium, adherence and study retention in ALS clinical trials will be explored. The goal is to learn from prior studies and identify modifiable factors in how studies are designed and conducted that can expedite therapy development for ALS.

C69 MODIFIABLE BARRIERS TO ENROLMENT IN AMERICAN ALS RESEARCH STUDIES

BEDLACK R[1], WICKS P[2], HEYWOOD J[2], KASARSKIS E[3]

[1]*Duke University, Durham, NC, United States,* [2]*Patients Like Me, Cambridge, MA, United States,* [3]*University of Kentucky, Lexington, KY, United States*

E-mail address for correspondence: bedla001@mc.duke.edu

Keywords: enrolment, recruitment, advocacy

Background: A surprisingly small percentage of patients with ALS (PALS) enrol in research studies. Analysis of previously published ALS trials identified no obvious 'trial factors' that influence enrolment. 'Doctor factors' and 'patient factors' may play more prominent roles, as seen in other fields. Indeed, a small survey of North American ALS Clinic directors identified several physician behaviors that might be modified to improve enrolment.

Objective: To identify specific patient factors responsible for low enrolment in research studies.

Methods: Two online patient surveys and a cost analysis of previously published ALS studies.

Results: Survey responders were predominantly educated white males. Most got information about research studies from the Muscular Dystrophy Association or the ALS Association websites and support groups. Half the responders did not recall being invited to participate in a study; of those who were asked, 75% agreed to participate. The top reason for participation was to help others. Among those who participated in a study, 70% said they were never asked to do so again. Responders were confused about multiple aspects of studies, including a need to donate blood or spinal fluid more than once, and the amount of out-of-pocket cost that would be incurred. The main reason for declining participation was concern about physical and financial burdens. Indeed, our cost analysis suggested that these can be substantial; between 1992-2006, PALS made a total of 1,093,655 study visits, contributed 6.6 million hours of effort to published studies and faced a gasoline cost alone of $5,401,317. Limitations of our work include the small number of responders and inability to validate their diagnoses.

Discussion and conclusions: The above research has identified potentially modifiable patient factors which may influence enrolment in ALS research studies. These include failure to know about open studies, confusion over study methodologies and goals, and concerns about physical and financial burdens. The ALS Research Group (ALSRG) has several projects underway to try and affect these, including development of educational slides for use at support group talks, an ALS Clinical Research Learning Institute to empower a small group of PALS and caregivers to be research educators and advocates, an online trial registry similar to the Army of Women being used to invite large numbers of patients and healthy controls into new breast cancer studies, a video to standardize study presentations to patients, ALSUntangled to investigate alternative and off-label options, expansion of ALS Clinics offering research into more rural areas, and development of home based outcome measures.

C70 RESULTS OF A CLINICAL TRIAL OF TALAMPANEL IN PATIENTS WITH ALS

SHEFNER J[1], MEININGER V[2], THE TALAMPANEL ALS STUDY GROUP

[1]*SUNY Upstate Medical University, Syracuse, NY, United States,* [2]*Hôpital de la Salpétrière, Paris, France*

E-mail address for correspondence: shefnerj@upstate.edu

Keywords: clinical trial, excitotoxicity, Talampanel

Background: Many studies have implicated glutamate mediated excitotoxicity as an important mechanism of cell death in ALS, and have suggested this pathway as a potential target for altering disease course. Evidence suggests that riluzole works in part by inhibiting glutamate release. Talampanel, a selective ampa receptor antagonist, showed promise in a phase II trial of 60 patients with ALS.

Objectives: To determine whether Talampanel is efficacious and safe in patients with ALS.

Methods: 559 patients with ALS were randomized to receive either placebo, 75 mg, or 150 mg daily. The primary outcome measure was change from baseline of the ALSFRS-R. Sample size was selected to provide 80% power to detect 20% decrease in deterioration slope. Secondary and exploratory outcome measures included survival, change in rate of decline of strength measured by manual muscle testing, and change in rate of decline of vital capacity. Multiple safety measures were also obtained.

Results: There were no significant differences in patient characteristics at baseline among the three groups. Subjects entered the study at a mean age of 55.9 years; 65% of subjects were male. 16.1% of subjects had bulbar onset; mean time from first symptom to study entry was 18.7 months. 83% of subjects were on riluzole while entering the study. The change in ALSFRS-R after 12 months of treatment was -12.4 pts in the placebo group, -13.1 points in the 75 mg/d group, and -12.6 pts in the 150 mg/d group. These differences were not statistically significant. Similarly, there were

no differences in survival, strength, or pulmonary function between groups. While patients receiving active treatment had more adverse events than those receiving placebo, dropout rate was similar across groups; 28.6% dropout in placebo, 31.2% in the 75 mg/d group, and 30.6% in the 150 mg/d group. Dizziness, somnolence, and ataxia were noted more frequently in the active treatment groups in a dose related manner.

Discussion and conclusions: Talampanel was not effective in altering disease progression in this trial. Given the neurological nature of the adverse events, as well as evidence from various animal models, it is apparent that the drug did in fact reach CNS targets. This study does not suggest that the glutamate pathway is a poor target for new therapies; however, pure ampa antagonists are unlikely to be of benefit.

C71 PHASE II STUDY OF PIOGLITAZONE IN ALS

LUDOLPH AC, KASSUBEK J, MEYER T, THE GERMAN ALS STUDY GROUP*

University of Ulm, Ulm, Germany

E-mail address for correspondence: albert.ludolph@rku.de

Keywords: study biological markers

Background: Because of independent positive results in ALS preclinical models, pioglitazone is seen as a potential candidate for human ALS therapy. We performed a double-blind placebo-controlled prospective study of pioglitazone as add-on therapy to standard therapy with riluzole in patients with ALS.

Methods: A total of 219 ALS patients were enrolled across 15 ALS centres, according to the revised El Escorial criteria (possible, probable (clinical or lab-supported) or definite ALS). Participants received pioglitazone (45mg/day) or placebo for 18 months. In addition, to commonly used primary and secondary outcome measures (survival, ALS FRS/R, quality of life) we measured pioglitazone blood levels and the effects of pioglitazone on cytokines and parameters of lipid metabolism, including quantitative MRI measurements, in a subgroup of participants.

Results: Interim analysis by the Data Monitoring Committee (DMC) determined the death rate in the pioglitazone group 27/109 (24.8%) compared with 18/110 (16.4%) in the placebo group. At the interim analysis point, 46 patients had completed the 18 month treatment period. In light of the interim analysis, it was considered extremely unlikely that the study would demonstrate significant therapeutic benefit of pioglitazone after the 18 month treatment period in the remaining trial participants.

Discussion: The study revealed that the application of pioglitazone to ALS-patients is safe. However, the results of this phase II study strongly suggest that pioglitazone is ineffective in slowing down the course of clinical ALS. We are presently performing detailed analyses of the data, including biological markers.

*AC Ludolph, Ulm; J Kassubek, Ulm; T Meyer, Berlin; T Grehl, Bochum; M Heneka, Bonn; A Storch, Dresden; J Weishaupt, Göttingen; S Zierz, Halle/Saale; S Petri, R Dengler, Hannover; GD Borasio, München; P Young, Münster; N Weidner, U Bogdahn, Regensburg; M Wittstock, R Benecke, Rostock; B Schrank, Wiesbaden; C Wessig, KV Toyka, Würzburg; J Grosskreutz, Jena

SESSION 10A GLIAL CELL BIOLOGY

C72 MECHANISMS AND THERAPY IN ALS: GLIA AS DETERMINANTS OF DISEASE PROGRESSION

CLEVELAND D

Ludwig Institute for Cancer Research, La Jolla, CA, United States, University of California, San Diego, La Jolla, CA, United States

E-mail address for correspondence: dcleveland@ucsd.edu

Since its description by Charcot in 1869, the mechanism of selective death of motor neurons in Amyotrophic Lateral Sclerosis (ALS) has remained elusive. An inherited form caused by mutation in superoxide dismutase (SOD1) triggers disease through an acquired toxicity unrelated to dismutase activity. Using mice carrying a deletable mutant gene or viral encoded siRNA to diminish mutant expression within motor neurons, disease onset is slowed but progression is not. Microhemmorages stemming from damage to the vasculature occur presymptomatically, albeit use of selective gene excision demonstrates this does not arise from mutant SOD1 acting within the endothelial cells of the capillaries. Reducing mutant SOD1 synthesis in two glial cell types, astrocytes or microglia, has little effect on disease onset, but strikingly slows disease progression. Reducing mutant synthesis in Schwann cells implicates a cascade of oxidative damage from the underlying axon to the Schwann cell as a component driving disease progression. Thus, toxicity is non-cell autonomous, with mutant SOD1 acting within motor neurons driving disease onset, while damage within neighboring glial (astrocytes and microglia) accelerates disease progression.

These findings validate therapies to slow disease progression that target astrocytes or microglia, including stem cell replacement or gene silencing approaches. One cell replacement therapy now underway is through injection of stem cell derived astrocytic progenitors. With discovery that peripherally administered Adeno Associated virus (AAV9) effectively transduces astrocytes after crossing the blood brain barrier, a final therapeutic approach now under development is use of AAV9-encoded shRNA to diminish mutant synthesis SOD1 within astrocytes.

Another third for targeting glial-driven SOD1-mediated ALS stems from administration after disease onset of Activated Protein C (APC), a signaling protease with anticoagulant and cytoprotective activities and which in the United States is already an FDA approved drug. APC is shown to cross the blood-spinal cord barrier where it activates Protease Activated Receptors 1 and 3 (PAR1 and PAR3) on the cell surfaces of motor neurons and microglia, thereby triggering transcriptional repression of SOD1 by inhibiting the Sp1 transcription factor. Delayed disease progression after APC administration supports its use as a therapy in ALS.

A final therapeutic approach targeted to glial cells exploits continuous infusion to deliver antisense oligonucleotides (ASOs). Infusion of such ASOs is shown to produce widespread gene silencing throughout the nervous system. Such ASOs catalytically target the mRNAs to which they hybridize for destruction by an endogenous, nuclear enzyme RNase H. Infusion of such an ASO to SOD1 slows SOD1 mutant-mediated disease progression, doubling disease duration. A clinical trial has now been initiated to test such ASO infusion as a treatment for SOD1-mediated inherited ALS.

C73 GENE EXPRESSION PROFILING OF ASTROCYTES IN PRE-SYMPTOMATIC G93A SOD1 MICE REVEALS DYSFUNCTION IN THE ASTROCYTE-MOTOR NEURONE LACTATE SHUTTLE

FERRAIUOLO L, HIGGINBOTTOM A, HEATH P, GREENALD D, KIRBY J, SHAW P

University of Sheffield, Sheffield, United Kingdom

E-mail address for correspondence: l.ferraiuolo@sheffield.ac.uk

Keywords: microarray, motor neurons, astrocytes

Background: Amyotrophic lateral sclerosis (ALS) is characterized by progressive death of upper and lower motor neurons (MN). Experiments on mice expressing mutant forms of the superoxide dismutase 1 (SOD1) enzyme have indicated that the development of the pathology requires interaction between MN and their non-neuronal neighbour, mainly astrocytes (1-2). Astrocytes play an important role in ALS, but the way in which the expression of mutant SOD1 *in vivo* alters their properties and their precise role in the generation of MN injury remains to be clarified.

Objectives: 1) To examine expression profile changes occurring within astrocytes in the spinal cord of G93ASOD1 mice at the presymptomatic stage of disease (60 days); 2) To identify pathways involved in the development of the neurodegenerative process at 60 days; 3) To validate with functional assays the involvement in the neurodegenerative process of the pathways identified through microarray analysis.

Methods: 1500 astrocytes were isolated using laser capture microdissection (LCM) from lumbar spinal cord sections stained with the astrocyte specific marker aldehyde dehydrogenase L1l (AldhL1l) (3). RNA was extracted, amplified and labelled and 15 µg cRNA was applied to the Affy Mouse 430 2.0 GeneChip. Transcription profiles of astrocytes from 3 60 day G93ASOD1 mice and 3 non-transgenic littermates were compared using ArrayAssist System 4.0 (Iobion). A threshold of fold change ≥ 2 plus P value ≤ 0.05 was used to identify differentially expressed genes.

Results: Initial comparison of the expression profile of astrocytes from 60 day G93ASOD1 mice and non-transgenic littermates showed a significant change in expression of 1109 genes. 526 transcripts are downregulated, and 583 are upregulated. The genes have been categorised according to their molecular function, and include genes involved in the transcription process, apoptosis, inflammation and metabolism. G93ASOD1 astrocytes show an increase in transcripts encoding for Ngf and several chemokines. One of the main features is an overall downregulation in genes involved in the carbohydrate metabolism and in particular the monocarboxylate transporter 5 (Mct5), in contrast to upregulation in the main genes involved in fatty acid synthesis and activation. Several transcripts involved in the WNT signalling cascade are also differentially expressed. This pathway is known to stimulate cell proliferation and cytoskeletal changes.

Discussion: Previous data obtained from the gene expression profile of MN isolated from SOD1G93A mice suggest that dysregulation of carbohydrate metabolism is one of the main features of the early stages of disease. Ngf upregulation has already been reported as potentially toxic for mature MN (4).

We aim to investigate further the reported dysregulation as potential mechanism leading to motoneuronal damage.

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C74 COMPREHENSIVE GENETIC ANALYSIS OF ADULT REACTIVE ASTROCYTES IN RODENT MODEL (SOD1 G93A) OF ALS REVEALS MASSIVE MOLECULAR REORGANIZATION *IN VIVO*

YANG Y[1], ROTHSTEIN J[2]

[1]*Department of Neuroscience, Tufts University School of Medicine, Boston, MA, United States,* [2]*Johns Hopkins University School of Medicine, Baltimore, MD, United States*

E-mail address for correspondence: yyang22@jhmi.edu

Keywords: RNA transcriptiome, reactive astrocyte, ALDH1L1

Background: Involvement of non-cell autonomous mechanisms has been demonstrated in the pathogenesis of ALS. In particular, astrocytes have been shown to significantly affect the disease progression, possibly by promoting the excitotoxicity through reduced glutamate uptake, altered metabolic support of neurons, and by secreting toxic factors that induces motor neuron death. However, the *in vivo* molecular mechanisms of astroglial dysfunction remain largely elusive, as embryonic/cultured astrocytes poorly reflect *in vivo* biology and until recently no reliable, accurate method was available to study *in vivo* astroglia.

Objective: This study aims to investigate the molecular changes in adult astrocytes, *in vivo*, during disease progression for a better understanding of the mechanisms/pathways of reactive astrocytes that may affect the pathogenesis/progression of ALS in rodent models.

Methods: Whole genome RNA transcriptome analysis was performed to investigate the molecular changes of astrocytes in diseases. Fluorescent activated cell sorting (FACS) and translating ribosome affinity purification approach (TRAP) approaches were employed to isolate the mRNA from astrocytes. For FACS approach, astroglial reporter mice GFAP eGFP or BAC ALDH1L1 eGFP were employed that selectively label astrocytes *in vivo*. Spinal cord astrocytes were isolated by FACS approach from GFAP eGFP X SOD1 G93A or BAC ALDH1L1 eGFP X SOD1 G93A mice at different ages (pre-symptomatic, symptomatic, and end stage). Total RNA was further isolated from these astrocytes and hybridized with mouse exon array for RNA transcriptome analysis. For TRAP approach, ribosomes of spinal cord astrocytes will be selectively labeled by an eGFP tag which allows specific pull-down of not only ribosomes but also the translating mRNA that binds to the ribosomes in astrocytes of BAC ALDH1L1 TRAP and BAC GFAP TRAP mice. This provides an alternative approach that purifies translating mRNA from spinal cord astrocytes without FAC sorting. These mice will be crossed with SOD1 G93A mice. Ribosomes and translating mRNAs will be isolated from BAC ALDH1L1 TRAP X SOD1 G93A or BAC GFAP TRAP X SOD1 G93A mice at different age (pre-symptomatic, symptomatic, and end stage), and will also be subject to the RNA transcriptome analysis.

Results: Various astroglial reporter and TRAP mice were characterized. We found that the newly identified astroglial marker ALDH1L1 expression (mRNA, protein, and promoter activity) is up-regulated in reactive astrocytes in SOD1 G93A condition. Initial RNA transcriptome analysis from the end-stage spinal cord indicates that expression levels of oligodendrocyte markers, including MAG, MOG, MOBP, MBP, and PLP are strongly induced in cells sorted from GFAP eGFP X SOD1 G93A mice.

Discussion and conclusions: Our initial results from RNA transcriptome analysis unexpectedly suggest that reactive astrocytes may lose their maturation identity. RNA transcriptome from other stages are ongoing for the overall gene expression change in astrocytes in SOD1 G93A spinal cord.

C75 MONOCARBOXYLIC TRANSPORTER (MCT1) BAC REPORTER AND OVER EXPRESSER MICE AS TOOLS TO REVEAL NOVEL GLIAL PATHOGENESIS IN ALS

LEE Y, JIN L, ROTHSTEIN J

Johns Hopkins University, Baltimore, MD, United States

E-mail address for correspondence: jrothstein@jhmi.edu

Keywords: astroglia, oligodendroglia, metabolism

Background: Monocarboxylate transporter (MCT) is the SLC16A family of membrane proteins that transport monocarboxylates such as lactate, pyruvate, and ketone bodies. Molecular MCT subtypes have unique tissue and cellular distribution with different kinetic properties. MCT-1 expressed in astrocytes has lower affinity for lactate than MCT-2 expressed in neurons, supporting the lactate shuttle hypothesis, in which lactate released from astrocytes is taken up by neurons as an energy substrate. However, there is no clear evidence which cell type of the central nervous system (CNS) expresses each MCT transporter *in vivo*. CNS energy demand is higher than other organs. Therefore, alteration of energy metabolism might be detrimental in many neurological diseases including amyotrophic lateral sclerosis (ALS). In preliminary experiments, MCT-1 expression is markedly reduced in ALS patients, and end stage of superoxide dismutase 1 (SOD-1) and mutant TDP-43ALS mouse models.

Objectives: We aimed at generating MCT-1 bacterial artificial chromosome (BAC) transgenic mouse and MCT-1 overexpression mouse models to better understand its expression and functions during the pathogenesis of ALS by cross-breeding with SOD-1 mice.

Methods: Mouse BAC RP23-208N5, containing the entire MCT-1 gene plus 50 kb upstream of the first exon and 132.2 kb downstream of the last exon, was modified using a double homologous recombination with the SV-RecA shuttle vector to insert a reporter gene, tdTomato. MCT-1 cDNA was inserted into human glial fibrillary acidic protein promoter (GFAP) driven internal ribosomal entry site (IRES) and EGFP sequences for its expression in astrocytes. The final modified MCT-1 BAC and GFAP-MCT-1-IRES-EGFP constructs were injected into mouse pronuclei for generation of transgenic mice.

Results: Surprisingly, tdTomato expresses predominantly in oligodendrocytes and specific type of neurons in brain and exclusively in oligodendrocytes in spinal cord. In addition, it expresses in blood, muscle, retina, lung, heart, liver, pancreas, spleen, stomach, intestine, colon, and kidney. Overexpression of MCT-1 was shown along with EGFP expression in astrocytes in the CNS. Interestingly, the most strong expression line showed clearly obese phenotype as well as aggressive

behavior. The other lines have same phenotype with variable level depending on MCT-1 expression.

Discussion and conclusions: Unexpected discovery of MCT-1 expression in oligodendrocytes introduces new metabolic coupling among astrocytes, oligodendrocytes, and neurons in the CNS. Although the rate of lactate utilization in cultured oligodendrocytes was much higher than astrocytes and neurons, lactate transport in oligodendrocytes has not been studied *in vivo*. Our finding in mice shows clearly the existence of MCT-1 in oligodendrocytes, suggesting the possible effects of oligodendrocytes on ALS pathogenesis by alteration of MCT-1 expression.

C76 FOCAL *IN VIVO* TRANSPLANTATION OF MUTANT SOD1 GRP-DERIVED ASTROCYTES INFLUENCES WILDTYPE MOTOR NEURON VULNERABILITY

PAPADEAS S, KRAIG S, O'BANION C, LEPORE A, MARAGAKIS N

Johns Hopkins University School of Medicine, Baltimore, MD, United States

E-mail address for correspondence: nmaragak@jhmi.edu

Keywords: glia, stem cells, cell autonomy

Background: Evidence from *in vitro* studies, transgenic mutant (mSOD1) rodents, chimeric mouse models, and conditional CRE-LOX systems have identified astrocytes as key drivers of motor neuron (MN) degeneration and disease progression in ALS and has underscored the importance of astrocyte-specific disease mechanisms in ALS. However, a major shortcoming using *in vivo* rodent models is that mSOD1 is expressed in all cell types and is anatomically ubiquitous. As a result, these animals display a rapidly progressive disease that requires extrapolation of relevant pathways on animal survival measured in days or weeks. Furthermore, the specific *in vivo* influences of mSOD1 astrocytes on wildtype motor neurons, astrocytes, and microglia have not been elucidated.

Objectives: By transplanting mSOD1 glial restricted precursors (GRPs) focally into defined wildtype rat spinal cord levels, we studied regional influences of mSOD1 astrocytes on other wildtype (WT) cell types (including WT motor neurons, astrocytes, and microglia) and the role of mSOD1 astrocytes in disease progression and anatomical spread.

Methods: GRPs overexpressing human $mSOD1^{G93A}$, wildtype (WT GRPs), human wildtype SOD1 (SOD^{WT}), dead cells, and media were transplanted in cervical spinal cord ventral gray matter in 90 day old WT rats and then followed *in vivo* for up to 3 months. The influence of mSOD1 on GRP survival, differentiation, anatomical localization, and migration were assessed.
Spatial interaction of transplanted $mSOD1^{G93A}$ GRP-derived astrocytes with host astrocytes and motor neurons (MNs) were examined, as well as pathohistological outcomes of $mSOD1^{G93A}$ GRP transplantation, including host astrocyte and MN survival, ubiquitin aggregation, host astrogliosis and microglial activation.
To assess behavioural influences of $mSOD1^{G93A}$ GRP-derived astrocytes, forelimb and hindlimb strength was assessed as we have described previously. We examined electrophysiological outcome by measuring phrenic compound muscle action potentials (CMAPs) following phrenic nerve stimulation.

Results: Animals were analyzed at 1 and 3 months following transplantation. We determined that $mSOD1^{G93A}$ does not influences astrocyte survival, migration, or proliferation but does influence the capacity for astrocyte differentiation. $SOD1^{G93A}$ GRP-derived astrocytes, but not WT or SOD^{WT} GRP-derived astrocytes, induced ubiquitin inclusions and death in host WT motor neurons. This was accompanied by ventral horn microgliosis. Interestingly, transplantation of $SOD1^{G93A}$ GRPs was sufficient to result in focal forelimb weakness and electrophysiological declines in phrenic nerve CMAPs which declined slowly over time and then become static.

Discussion and conclusions: These findings suggest that the presence of mutant SOD1 in astrocytes has influences on astrocyte differentiation and also induces wildtype motor neuron pathology, motor neuron cell death, and a decline in behavioral and electrophysiological functions over a period of months which subsequently becomes static. These pathological findings are accompanied by microgliosis. Taken together, these data provide *in vivo* evidence that $mSOD1^{G93A}$ astrocytes alone are sufficient to induce motor neuron vulnerability and functional motor decline.

SESSION 10B HOLISTIC CARE

C77 PRACTICAL IMPLICATIONS OF GENETICS IN ALS FOR PATIENTS AND FAMILIES

SIDDIQUE N

Northwestern University, Feinberg School of Medicine, Chicago, IL, United States

E-mail address for correspondence: nsiddique@northwestern.edu

Keywords: genetic testing, genetic counselling, inherited ALS

About 10% of ALS is known to be inherited, while the remaining 90% occurs as a single case within a family (sporadic ALS). Causative genes, including SOD1, TDP-43, FUS-TLS and others, have been identified for roughly 30% of inherited cases. Several genes, including the PON cluster, have also been associated with sporadic ALS. This explosion of information has made it difficult for both health care providers and families to determine its relevance for a given family. This session will review very basic genetics, delineation of sporadic and familial disease, genes currently known to cause ALS, genes associated with ALS. There will be recommendations for circumstances in which to do genetic testing and detailed discussion of implications for genetic testing in both patients and family members.

C78 TELEMEDICINE TO FACILITATE ALS RESEARCH

GRONKA S[1], WALKER M[1], WUU J[1], STANISLAW C[1], FANOS J[1], ATASSI N[2], JAFFA M[2], ANDERSEN P[3], CUDKOWICZ M[2], BENATAR M[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Massachusetts General Hospital, Boston, MA, United States,* [3]*Umea University, Umea, Sweden*

E-mail address for correspondence: michael.benatar@emory.edu

Keywords: telemedicine, remote evaluations, familial ALS

Background: Telemedicine, derived from the Greek 'tele' (at a distance) and the Latin 'medicine' (healing), refers to the use of telecommunication and other methods to perform remote medical evaluations. Telemedicine has the potential to enhance clinical research in a rare disease such as ALS and an ultra-rare disease such as familial ALS. It may improve recruitment by reducing the burden of travel for study participants and expanding the geographic scope and population from which participants may be recruited. It may also reduce costs.

Methods: Genetic counseling data were derived from the Pre-familial ALS (Pre-fALS) study, which enrolls healthy individuals at genetic risk for fALS. Participants who elect to undergo genetic testing with disclosure of results were randomized to receive pre- and post-test counseling either via telephone (TL) or in-person (IP). Respiratory muscle function is evaluated and blood collected remotely in the Arimoclomol trial, which enrolls affected individuals with fALS. Respiratory muscle function is evaluated using both forced expiratory volume in 6 seconds (FEV6), which can easily be measured in the home, and slow vital capacity (SVC), which is measured in the clinic. Blood for safety laboratory evaluations was collected in homes of Arimoclomol patients and analyzed in a central laboratory with results communicated via web and fax to the study center.

Results: Of the first 30 Pre-fALS participants who have elected to learn their genetic results, 15 have been randomized to each counseling group. Five TL and 3 IP subjects were SOD1+; the others were SOD1-. Fourteen subjects (3 SOD1+ and 4 SOD1- from each counseling group) were randomly selected for semi-structured interviews to formally assess TL and IP counseling. Among these 14 subjects, only 3 (2 TL and 1 IP) expressed preference to have received the other counseling modality. Regardless of positive or negative results, participants reported that they had adapted well to receiving results. Sixteen Arimoclomol patients yielded FEV6 and SVC data from 27 visits. Reproducibility of SVC and FEV6 were comparable with intraclass correlation coefficients of 0.98-0.99. There was high correlation between SVC and FEV6 expressed in liters ($r=0.97$, $P<0.001$) and percent predicted ($r = 0.92$, $P < 0.001$). In addition, all 64 remote blood collections in the homes of the 16 patients occurred within the designated visit window with no failed/missed collections.

Discussion: Pre-symptomatic genetic counseling was safely and effectively performed via telephone. Respiratory muscle function was reliably measured and blood was collected for safety laboratory tests, all without the need for participants to leave home. The use of these remote evaluations as part of a telemedicine approach has greatly facilitated our ability to undertake an observational study in people at risk for fALS and a clinical trial in patients already affected by fALS.

C79 IDENTIFYING THE NEEDS OF THE ALS/FTD CAREGIVER: DEVELOPMENT, IMPLEMENTATION, AND EVALUATION OF A CLINICAL ASSESSMENT INSTRUMENT

BROTHERS A[1], WALSH S[2], LYTER J[2], HOUSEMAN G[3], KLAPPER J[3], SIMMONS Z[1]

[1]*Penn State College of Medicine/Hershey Medical Center, Hershey, PA, United States,* [2]*ALS Association, Greater Philadelphia Chapter, Hershey, PA, United States,* [3]*ALS Association, Greater Philadelphia Chapter, Philadelphia, PA, United States*

E-mail address for correspondence: abrothers@hmc.psu.edu

Keywords: frontotemporal dementia, caregiver needs, caregiver assessment

Background: Despite estimates that cognitive-behavioral change occurs in up to half of people with ALS, there are currently no guidelines for the clinical management of this subset of patients. ALS with frontotemporal dementia (ALS/FTD) is accompanied by unique clinical concerns, and the services provided to caregivers will require individualization. In order for ALS treatment teams to tailor their interventions accordingly, it will be important to accurately assess caregivers' needs.

Objectives: 1) Understand the needs of family caregivers of patients with ALS/FTD; 2) Develop a questionnaire to identify caregiver concerns related to cognitive-behavioral changes; 3) Evaluate the assessment instrument for ease of completion and perceived usefulness.

Methods: An evidence-based practice approach was used to develop an ALS/FTD caregiver assessment instrument using three types of input: 1) comprehensive literature review; 2) expert clinician opinion from members of a multidisciplinary

ALS clinic team; 3) ALS/FTD caregiver opinion through structured telephone interviews with six family caregivers. A process evaluation took place to gather caregivers' feedback regarding the assessment instrument. Open-ended responses from the caregiver questionnaire and the process evaluation were qualitatively analyzed using a thematic analysis approach. This study was approved by the Penn State Hershey Medical Center Institutional Review Board.

Results: The assessment instrument consisted of 12 questions regarding patient safety concerns, financial and legal matters, and caregiver emotional well-being. Eight caregivers of patients with ALS/FTD completed the assessment. There were 4 men and 4 women, all spouses. All reported patient safety concerns, most commonly 'Ability to find way home if lost' (6 caregivers); 'Risk of falling' (6); 'Taking medications as prescribed' (5); and 'Ability to be left alone or unsupervised' (5). Half of the caregivers reported concerns related to the patient's financial behaviors. Six reported that the patient had designated a healthcare power of attorney, and five noted that an advance directive had been completed. Half reported feeling at least moderately burdened. Caregivers reported no difficulty in completing the questionnaire; 100% indicated that they thought the care they received from the ALS team would improve as a result of completing the questionnaire. The most commonly repeated qualitative themes related to caring for someone with ALS/FTD included a) sleep difficulties (patient and caregiver); b) desire for candid information about prognosis; and c) the difficulty of managing the ALS along with FTD symptoms.

Discussion and conclusions: Despite a small sample size, preliminary analyses show the ALS/FTD caregiver assessment to be a useful clinical instrument. It will be revised according to caregiver feedback, and implemented in the ALS Clinic for continued data collection. The results of this study will be used to design a psycho-educational intervention session for caregivers, as well as to develop a repertoire of individualized interventions for caregivers of patients with ALS/FTD.

C80 IDENTIFYING NEEDS AND INTERVENTIONS TO SUPPORT CAREGIVERS OF PATIENTS WITH ALS

BREMER B[1], STEPHENS HE[2], WALSH S[3], FELGOISE S[4], SIMMONS Z[2]

[1]*Penn State Harrisburg, Harrisburg, Pennsylvania, United States,* [2]*Penn State College of Medicine, Hershey, Pennsylvania, United States,* [3]*Greater Philadelphia Chapter, ALS Association, Philadelphia, United States,* [4]*Philadelphia College of Osteopathic Medicine, Philadelphia, United States*

E-mail address for correspondence: hstephens1@psu.edu

Keywords: caregiving, assessment, interventions

Background: As ALS progresses, an increasing burden is placed on caregivers to assist in activities of daily living and use of medical equipment, and to provide emotional support. Much of this care and support is provided by family members who must concurrently face the emotional strain of the impending loss of a loved one.

Objectives: A team of health care professionals and academic researchers used the evidence based practice approach to develop a comprehensive list of caregiving tasks, supportive activities, factors affecting caregiver physical and psychological health, and caregiver confidence, rewards, and concerns. These were incorporated into a Caregiver Assessment Questionnaire with both closed and open ended items which was mailed to caregivers.

Results: 102 caregivers of patients seen at a multidisciplinary outpatient ALS clinic in a four month period completed the questionnaire (65% women; 91% Caucasian, 78% spouses). Mean (SD) age was 58.9 (12.7) years. A mean (SD) of 14.79 (9.69) hours caregiving daily was reported. 17 concerns about caregiving tasks (0 none, 5 high) were combined (Chronbach's alpha 0.94) to produce a mean (SD) task concern score of 1.92 (1.17), range 0.12 to 4.88. Concern ratings ranged from a high related to providing emotional/spiritual support (mean 2.40) and injury prevention (mean 2.35) to lows related to medications (mean 1.47) and feeding (mean 1.21). Fear about the future was a common theme identified from the qualitative data. The supportive activity most commonly cited by caregivers was socializing (78.8%), followed by journaling/reading (50%) and spiritual practices (48%). Least likely to be reported as helpful were ALS education (1.0%), counseling, and support groups (6.7% each). Activities frequently discontinued while caregiving included socializing and hobbies. Love and character strengths were frequently cited as important for caregiving. 13.5% were positive on a depression screen, and 36.6% on a stress screen.

Discussion: 1) Specific concerns of ALS caregivers can be identified, with injury prevention, providing emotional and spiritual support, and fear of the future being among the top ones; 2) Support strategies for ALS caregivers most commonly include socializing, journaling or reading, and spiritual practices; 3) ALS caregivers often abandon supportive tasks such as socializing and hobbies; 4) Stress and depression are not uncommon among ALS caregivers. The use of a caregiver survey is one intervention that can be used to assess caregiver needs and guide support.

C81 A RANDOMIZED CONTROLLED TRIAL OF PHYSICAL ACTIVITY IN INDIVIDUALS WITH ALS: PRELIMINARY EXPERIENCE AND SHORT-TERM FOLLOW-UP RESULTS OF TREATMENT WITH DIFFERENT EXERCISE PROTOCOLS

LUNETTA C[1], SANSONE V[2], MAESTRI E[1], BELLA P[1], BETTINELLI M[1], PANZERI M[2], CELLOTTO N[1], MEOLA G[2], CORBO M[1]

[1]*NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Niguarda Ca' Granda Hospital, Milano, Italy,* [2]*Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy*

E-mail address for correspondence: christian.lunetta@centrocliniconemo.it

Keywords: physical activity, cycloergometer, ALSFRS-R

Background: Physical exercise exerts a wide range of benefits on an organism's overall health and well-being. Exercise contributes positively toward an individual's healthy weight, muscle strength, immune system, and cardiovascular health. In addition, exercise has also been shown to be neuroprotective in both the central and peripheral nervous systems. Whether physical activity promotes or prevents progression of motor neuron degeneration in ALS is still debated. Vigorous exercise exacerbates the disease progression in ALS animal models and patients whereas moderate exercise regimens improve ALS patients' functional scoring and disease symptoms.

Objective: 1) To evaluate the effects of active exercise program associated with cycloergometer exercise compared to only active or passive exercise programs on disease progression; 2) To define the impact of the different exercise programs on the patient's quality of life.

Patients and methods: Patients were randomized to receive active exercise program associated with cycloergometer activity (group A) or only active (group B) or passive (group C) excercise programs. Moreover, all patients were trained to home-based passive exercise programs. At baseline and after 2, 4, and 6 months, patients were assessed by manual muscle strength testing (MMT), ALSFRS-R, Forced Vital Capacity percentage (FVC%), and McGill Quality of Life (MGQoL) questionnaire.

Results: Sixteen ALS patients were screened and randomly assigned to one of three (group A, n= 5; group B, n= 5; group C, n= 6). Two patients did not complete the trial (one of group B and C, respectively). At 4 months, the group A had significantly higher ALSFRS-R score (A 36.5±0.7; B 29±2.8; C 33.5±2.1) and lower MGQoL physical symptoms subscale score (A 11±4.6; B 22.7±6.4; C 21.5±9.2) compared to the other two groups. No adverse events related to the intervention occurred, MMT and FVC indicated no negative effects.

Discussion: The patients treated with an active exercise program associated with cycloergometer activity demonstrated less motor deterioration and improvement of physical symptoms, as showed by ALSFRS-R score and MGQoL respectively, compared to the other two groups. Compliance with home-based exercise programs is a common problem, especially in old and disabled people. Effects of exercise on most examined parameters are probably short-lived if the program is not continued.

Conclusions: Our study, although small and short, showed that the active exercise program associated with the use of cycloergometer had significantly better function, as measured by ALSFRS-R and quality of life score without adverse effects as compared with subjects receiving exclusively active or passive exercise programs.

SESSION 11A CELL BIOLOGY AND PATHOLOGY

C82 NOVEL RNA BINDING PROTEINS IN ALS

GITLER AD

Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA, United States

E-mail address for correspondence: gitler@mail.med.upenn.edu

Keywords: RNA binding proteins, TDP-43, Ataxin-2

Amyotrophic lateral sclerosis (ALS) is a devastating human neurodegenerative disease. The causes of ALS are poorly understood, although the protein TDP-43 has been suggested to play a critical role in disease pathogenesis. Here we show that Ataxin-2, a polyglutamine (polyQ) protein mutated in spinocerebellar ataxia type 2 (SCA2), is a potent modifier of TDP-43 toxicity in animal and cellular models. The proteins associate in a complex that depends on RNA. Ataxin-2 is abnormally localized in spinal cord neurons of ALS patients. Likewise, TDP-43 shows mislocalization in SCA2. To assess a role in ALS, we analyzed the Ataxin-2 gene (ATXN2) in 915 ALS patients. We found intermediate-length polyQ expansions (27-33 Qs) in ATXN2 significantly associated with ALS (4.7% of cases, $P=3.6\times10^{-5}$). These data establish ATXN2 as a new and relatively common ALS disease susceptibility gene. Further, these findings indicate that the TDP-43/Ataxin-2 interaction may be a promising target for therapeutic intervention in ALS and other TDP-43 proteinopathies.

C83 MECHANISMS OF ALS RESISTANCE IN MOTOR NEURONS OF OCULOMOTOR AND ONUF'S NUCLEI

KAPLAN A[1], TOWNE C[2], SPILLER K[1], KANNING K[1], AEBISCHER P[2], HENDERSON C[1]

[1]*Columbia University, Center for Motor Neuron Biology and Disease, New York, United States,* [2]*Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland*

E-mail address for correspondence: ch2331@columbia.edu

Keywords: oculomotor, resistance, gene profiling

Background: ALS patients retain eye movement and voluntary control of continence until terminal stages of the disease. This reflects the pathological observation that motor neurons in the oculomotor, trochlear, and abducens nuclei, which innervate extraocular muscles (EOMs), and motor neurons of Onuf's nucleus, which innervate pelvic sphincters, are mostly spared. If even a fraction of this resistance could be conferred on vulnerable spinal motor neurons, the benefits for patients would be significant.

Objectives: We wish to determine the molecular basis of ALS resistance in oculomotor and Onuf's nuclei as a strategy for identifying novel therapeutic targets.

Methods: Microarray analysis was performed on motor neuron populations isolated using laser capture microdissection from wild type P7 mice. MMP-9 expression was validated by *in situ* hybridization and immunostaining. Effects of MMP-9 on neuronal survival were quantified using ES-cell derived motor neurons (ES-MNs).

Results: A similar pattern of disease resistance was found in SOD1G93A mice. By endstage, only 13% of endplates in the fast *tibialis anterior* muscle were innervated. In marked contrast, this figure was nearly 100% in EOMs, and 80% in sphincter muscles. To uncover intrinsic molecular properties common to resistant nuclei, gene expression profiles of motor neurons in the oculomotor nucleus and the L6 DL nucleus (the homologue of Onuf's nucleus) of wildtype P7 mice were compared to that of vulnerable L5 lumbar motor neurons. Counting only >10-fold differences, nearly 100 genes were differentially expressed in either oculomotor vs. L5, or DL vs. L5. Of these, 17 genes were co-regulated in both resistant populations. One such gene was MMP-9 (matrix metalloproteinase-9), which is strongly expressed by many spinal and cranial motor neurons throughout postnatal life but is absent from oculomotor and DL nuclei. Even within disease-susceptible pools, a strong correlation exists between the fraction of motor neurons that initially express MMP-9 and the fraction lost at endstage in SOD1-G93A mice. Indeed, the ~50% of motor neurons in these pools that survive to endstage do not express MMP-9. *In vitro,* activated MMP-9 (but not other MMPs) triggered death of motor neurons. MMP-9 levels have been reported to be elevated in ALS patients. Our data raise the possibility that motor neuron-derived MMP-9 may contribute to degeneration of vulnerable motor neurons in ALS, and that its absence may be one cause of resistance in oculomotor and Onuf's nuclei.

Discussion and conclusions: A set of molecular differences common to disease-resistant motor neurons distinguishes them from the majority of vulnerable motor neurons. These may constitute potential therapeutic targets but their functional role *in vivo* remains to be determined. We are currently testing the effects of MMP-9 knockdown and pharmacological inhibition on disease progression in SOD1G93A mice.

C84 INTERCELLULAR MISFOLDING OF HUMAN WTSOD1 PROPAGATED *IN VITRO*

CASHMAN N, GRAD L, YANAI A

University of British Columbia, Vancouver BC, Canada

E-mail address for correspondence: neil.cashman@vch.ca

Keywords: SOD1, protein misfolding, prion-like activity

Background: Motor neuron cell death in ALS progresses through the neuraxis in a spatiotemporal manner reminiscent of prion disease. We have previously shown that expression of G127X and G85R, misfolded human mutants of superoxide dismutase-1 (SOD1), can induce misfolding of natively-structured wild-type SOD1 in human mesenchymal and neural cell lines, as determined by molecular surface immunoreactivity with misfolding-specific monoclonal antibodies (mAbs) and acquisition of protease sensitivity (suggesting structural loosening). Mouse wtSOD1 did not support the conformational conversion reaction, consistent with a structure-sensitive 'species barrier'.

Objectives: To determine if misfolded human wtSOD1 is capable of supporting the SOD1 conformational conversion activity we have previously observed with mutant SOD1.

Methods: We transgenically over-expressed human wtSOD1 in HEK cells and monitored: 1) immunoreactivity with SOD1 disease-specific epitope (DSE) mAbs 3H1 (recognizing the misfolded electrostatic loop) and 10C12 (recognizing the

oxidized C-terminal dimer interface domain); 2) proteinase K (PK) sensitivity; 3) incorporation into non-native disulfide-linked oligomers and multimers; and 4) generation of reactive oxygen species (ROS), as determined by immunodetection of protein carbonylation. Furthermore, we ascertained whether misfolding of wtSOD1 could be transmitted between cells via supernatant transfer from transfected to naïve HEK cells.

Results: Transfection-driven overexpression of human wtSOD1 in HEK cells was accompanied by detectable SOD1 misfolding, as monitored by DSE immunoreactivity, marked enhancement of PK sensitivity, formation of non-native disulfide bonds and generation of ROS. Misfolding of human wtSOD1 was transmitted to untransfected HEK cells by incubation in conditioned media from wtSOD1 transfected HEK cells. No misfolded human SOD1 was detected in mouse N2a cells incubated in transfected HEK media, and knockdown of endogenous SOD1 expression in human HEK cells by siRNA abrogated the effect, consistent with a concentration- and structure-dependent SOD1 conformational conversion driven by misfolded human wtSOD1. Pre-incubation of wtSOD1-transfected conditioned media with poly-specific SOD1 antibodies or DSE mAbs also blocked the SOD1 conformational transducing activity.

Discussion: Our data are consistent with intercellular exportation, uptake and conformational conversion activity of misfolded wtSOD1, similar to that observed for the natural SOD1 mutants G127X and G85R.

Conclusion: Misfolded wtSOD1 participates in a template-directed misfolding cascade which can be transmitted between cells, providing a possible SOD1 misfolding-dependent molecular mechanism for propagation of sporadic ALS in the nervous system.

C85 ALS MUTATIONS IN FUS ARE CLUSTERED IN THE NUCLEAR LOCALIZATION SEQUENCE AND INDUCE STRESS GRANULES

GAL J, ZHANG J, KWINTER D, ZHAI J, JIA H, JIA J, ZHU H

University of Kentucky, Lexington, KY, United States

E-mail address for correspondence: haining@uky.edu

Keywords: FUS/TLS, nuclear localization sequence (NLS), stress granules

Mutations in FUS have been reported to cause a subset of familial amyotrophic lateral sclerosis (ALS) cases. Wild-type FUS is mostly localized in the nuclei of neurons, but the ALS mutants are partly mislocalized in the cytoplasm and can form inclusions. Little is known about the regulation of FUS subcellular localization or how the ALS mutations alter FUS function. Here we demonstrate that the C-terminus of FUS constitutes an effective nuclear localization sequence (NLS) as it targeted beta-galactosidase (LacZ, 116 kDa) to the nucleus. Deletion of or the ALS point mutations within the NLS caused cytoplasmic mislocalization of FUS. Moreover, we identified the poly-A binding protein (PABP1), a stress granule marker, as an interacting partner of FUS. PABP1 formed large cytoplasmic foci that co-localized with the mutant FUS inclusions. No such foci, which resemble stress granules, were observed in the presence of wild-type FUS. In addition, processing bodies, which are functionally related to stress granules, were adjacent to but not co-localized with the mutant FUS inclusions. Our results suggest that the ALS mutations in the C-terminal NLS of FUS can impair FUS nuclear localization and induce cytoplasmic mislocalization, inclusion formation, and potential perturbation of RNA metabolism.

C86 MUTANT FUS PROTEINS THAT CAUSE AMYOTROPHIC LATERAL SCLEROSIS INCORPORATE INTO STRESS GRANULES

BOSCO D[1], LEMAY N[1], KO HAE K[1], ZHOU H[1], BURKE C[1], KWIATKOWSKI T[2], SAPP P[1], MCKENNA-YASEK D[1], BROWN R[1], HAYWARD L[1]

[1]*University of Massachusetts Medical School, Worcester, MA, United States,* [2]*Massachusetts General Hospital, Boston, MA, United States*

E-mail address for correspondence: Lawrence.Hayward@umass-med.edu

Keywords: stress granules, FUS, zebrafish

Background: Dominant mutations in the nucleic acid binding protein FUS (*fused in sarcoma*), also known as TLS or heterogeneous nuclear ribonucleoprotein (hnRNP) P2, cause ~5% of familial ALS cases. FUS is a ubiquitously expressed protein normally concentrated in cell nuclei that has putative functions in DNA repair, transcription, and pre-mRNA splicing. In addition, FUS accompanies mRNA transcripts to the cytoplasm as a component of RNP granules in neurons and may influence the transport or translational regulation of specific mRNAs in dendrites. Initial clues suggest that the mutant variants of FUS may be localized abnormally in the cytoplasm of motor neurons under pathological conditions, but the mechanism(s) by which expression of the mutants injures motor neurons is not known.

Objectives: More than 15 ALS missense mutants are clustered within a non-classical nuclear localization signal (NLS) region that spans residues Pro508-Tyr526 at the C-terminus, while a truncation mutant (R495X) that causes a more aggressive phenotype removes the final 32 residues of FUS, including the NLS. We therefore tested whether ALS-linked FUS variants i) mislocalize upon expression in cell cultures and zebrafish embryos and ii) exhibit a mutant-specific phenotype in response to cellular stresses.

Methods: We engineered stable HEK-293 cell lines that express doxycycline-inducible GFP-tagged FUS constructs and also expressed human FUS variants in zebrafish embryos. Constructs encoding wild type or mutant FUS were injected into the yolk sac at the 1-2 cell stage, and the protein was detected at 24-72 h post fertilization either by live embryo imaging or by immunostaining using an anti-GFP antibody.

Results: We observed a striking redistribution from the nucleus into punctate cytoplasmic granules in both HEK-293 cells and zebrafish spinal cord for the clinically severe mutant (R495X) and an experimental truncation mutant (G515X). Quantitative measurements of GFP fluorescence intensity in live cells showed that the cytoplasmic:nuclear expression ratio per unit volume was significantly increased by 2-fold for GFP-R521G, by 29-fold for GFP-R495X, and by 48-fold for GFP-G515X in comparison to that for GFP-WT FUS. Furthermore, in response to translational arrest induced either by oxidative stress or by heat shock, the FUS mutants, but not WT FUS, assembled within minutes into larger perinuclear stress granules in proportion to their extent of cytoplasmic accumulation. In the case of heat shock, the focal accumulation of mutant FUS was partially reversible upon return to physiological temperature.

Discussion and conclusions: We report a novel ALS truncation mutant (R495X) that causes a relatively severe ALS phenotype compared to FUS missense mutations. Our findings also demonstrate a potential link between FUS mutations and cellular pathways involved in stress responses that may be relevant to altered motor neuron homeostasis in ALS.

C87 THE H63D HFE GENE VARIANT REDUCES SURVIVAL AND DISEASE DURATION IN SODG93A MICE

NANDAR W, NEELY E, SIMMONS Z, CONNOR J

Penn State University, College of Medicine, Hershey, PA, United States

E-mail address for correspondence: wnandar@hmc.psu.edu

Keywords: iron, H63D HFE, SODG93A/HFE+/H67D

Background: There is considerable interest in identifying a genetic basis for amyotrophic lateral sclerosis (ALS). The H63D HFE gene variant has been identified as present at higher frequency in ALS patients, and a meta-analysis indicates that its presence increases the risk of ALS 4-fold. At the cellular level, H63D mutations are associated with iron overload, increased oxidative stress, increased glutamate release, tau phosphorylation and ER stress, each of which is under investigation as contributing to ALS. We have proposed that H63D mutations establish a permissive milieu for ALS pathogenic factors.

Objectives: To determine whether the H63D gene variant is associated with ALS pathogenesis using the SOD1 mouse model of ALS.

Methods: We crossed the SOD^{G93A} mice with H67D mice (homologous to H63D mutation in humans) to generate a mouse line ($SOD^{G93A}/HFE+^{/H67D}$), which is heterozygous for the H67D gene variant and carries the SOD^{G93A} mutation. We determined the survival, disease duration, age at disease onset and body weight loss in $SOD^{G93A}/HFE+^{/H67D}$ mice. Disease onset was determined by monitoring the motor performance on a rotarod. Death was defined as the inability of the animal to right itself within 30 s after being placed on its side. Disease duration was the mean time from age at onset to death.

Results: We found that the survival of female $SOD^{G93A}/HFE+^{/H67D}$ mice (128.3±1.9 days) was significantly reduced when compared with female SOD^{G93A} mice (138.2±4.9 days). Female $SOD^{G93A}/HFE+^{/H67D}$ mice (18.07±1.4 days) also exhibited significantly shorter disease duration than SOD^{G93A} mice (33±5.9 days). The survival and disease duration of $SOD^{G93A}/HFE+^{/H67D}$ males (126.8±1.6; 22.82±3.2 days) was not different from male SOD^{G93A} mice (123.3±1.6; 24±6.5 days). Age at disease onset and body weight loss in both male and female $SOD^{G93A}/HFE+^{/H67D}$ mice were not significantly different from SOD^{G93A}mice.

Discussion and conclusions: The H63D HFE gene variant reduces the survival and disease duration in a transgenic mouse model of ALS. The epidemiological data, cell model based data and now animal model data all support HFE genotype as a factor in ALS pathophysiology. The shorter survival and disease duration observed in female $SOD^{G93A}/HFE+^{/H67D}$ mice indicates that the effect of the H63D HFE gene variant on ALS may be gender-dependent, analogous to gender-dependent differences in ALS incidence in humans in some age groups. We now have an animal model to evaluate the pathogenic mechanisms by which an HFE gene variant contributes to the course of ALS, and to test how intervention strategies may be impacted by HFE genotype. Because the H63D gene variant occurs in as many as 30% of ALS patients, the mouse model presented here has meaningful implications for human disease.

C88 P-TDP-43 INCREASE IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IS ASSOCIATED WITH CELLULAR STRESS: ROLE OF MITOCHONDRIA

AYALA V[1], NAUDÍ A[1], GRANADO A[1], CARABALLO-MIRALLES V[2], LLADÓ J[2], CACABELOS D[1], BOADA J[1], PAMPLONA R[1], PORTERO-OTÍN M[1]

[1]*Department of Experimental Medicine, University of Lleida-IRBLLEIDA, Lleida, Spain,* [2]*Department of Biologia/IUNICS, University of Illes Balears, Palma de Mallorca, Spain*

E-mail address for correspondence: victoria.ayala@mex.udl.cat

Keywords: phosphorylated TDP-43, oxidative damage, excitotoxicity

Background: Excitotoxicity plays an important role among the multifactorial mechanisms that lead to the death of motor neurons in ALS. As described with superoxide dismutase-1 and other proteins (eg ubiquitin), TAR-DNA-binding protein-43 (TDP-43) is aggregated in the cytoplasm in both the sporadic and SOD-associated forms of ALS. This abnormal localization can be associated to a lack of function of this protein in the nucleus, but the mechanisms involved in its mislocalization are unknown.

Objectives: Previous results in our laboratory suggest that excitotoxicity leads to an increase protein oxidation and endoplasmic reticulum stress contributing to motoneuron death in sporadic ALS (sALS). Hereby, we have examined what is the status of TDP-43 in a model of excitotoxic motor neuron death, reproducing features of sALS, and what could be the contributing factors for this mislocalization.

Methods: Excitotoxicity was induced by exposure to treohydroxyaspartate (THA) in the postnatal rat lumbar spinal cord organotypic cultures (1). We also used Neuro2a and SHSY-5Y cell lines as neuronal models. We measured content and distribution of TDP-43, p-TDP-43 (pS403/404 and pS409/410), ubiquitin and SMI-32 proteins, as well as typical ER and oxidative stress markers by Western blot, gas chromatography mass spectrometry (GC/MS) and immunochemistry techniques, as well as high resolution respirometry for mitochondrial function assessment.

Results: We show that p-TDP-43 is increased in a chronic excitotoxicity model along with the presence of fragments (≈25 kDa) and other hyperphosphorylated species, which have been previously detected in human ALS. Confocal measurements demonstrate that nuclear TDP-43 immunoreactivity decrease in the positive SMI-32 motoneurons after THA treatment. We also observed increase in both ubiquitin and phosphorylation of eukaryotic initiation factor 2 α (eIF2α) by confocal microscopy as well as profound changes in lipid composition and protein oxidative damage markers under excitotoxicity, associated to changes in oxygen consumption suggesting defects in mitochondrial respiratory complexes. We also demonstrate that ER and oxidative stress lead to increased cytosolic location of TDP-43 with implication of ERK1 and ERK2. Finally, we provide evidence that mitochondrial impairment induces TDP-43 phosphorylation and mislocalization.

Discussion and conclusions: We have previously shown that chronic excitototoxicity in organotypic spinal cord cultures, an established model of sALS, leads to ER stress, ubiquitin alterations, protein oxidative damage and changes in fatty acid profile (2). Here, we demonstrate that a mitochondrial impairment could contribute to both factors, finally leading to an increase in cytoplasmatic TDP-43 and aggregate formation associated to oxidative and ER stress in a model of excitotoxic motor neuron death.

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C89 REPETITIVE NERVE STIMULATION TRANSIENTLY OPENS THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE IN MOTOR NERVE TERMINALS OF SYMPTOMATIC MUTANT SOD1 MICE

NGUYEN K, BARRETT J, GARCÍA-CHACÓN L, DAVID G, BARRETT E

University of Miami Miller School of Medicine, Miami, FL, United States

E-mail address for correspondence: ebarrett2@med.miami.edu

Keywords: motor nerve terminal, mitochondria, permeability transition pore

Background: Mitochondria in motor nerve terminals temporarily sequester large Ca^{2+} loads during repetitive stimulation. In motor terminals of wild-type mice this Ca^{2+} uptake produces a small (~1-2 mV), transient depolarization of the mitochondrial membrane potential (Ψ_m). This Ψ_m depolarization increases ~5-fold in presymptomatic SOD1-G93A and -G85R mice, models of familial ALS (1).

Objectives: Investigate mechanisms underlying mitochondrial dysfunction in motor neurons of older, symptomatic mutant SOD1 mice by measuring stimulation-induced changes in Ψ_m depolarization in motor nerve terminals.

Methods: Mitochondria in levator auris longus neuromuscular preparations from symptomatic SOD1-G93A and -G85R mice were loaded with a potentiometric fluorescent dye, rhodamine-123 (Rh123). Changes in Rh123 fluorescence, indicating changes in Ψ_m, were measured in motor terminals stimulated with 3 successive trains of action potentials (100 Hz, 5 s trains separated by 30 s intertrain interval, neuromuscular transmission blocked with d-tubocurarine).

Results: Stimulation-induced Ψ_m depolarizations attain much higher amplitudes in motor terminals of symptomatic mutant SOD1 mice compared to presymptomatic mice. These large Ψ_m depolarizations decay more slowly and increment with successive stimulus trains. Additional Ψ_m depolarizations occur that are not synchronized with stimulation. These large Ψ_m depolarizations are reduced a) by cyclosporin A (CsA, 1 μM), which inhibits opening of the mitochondrial permeability transition pore (mPTP), or b) by replacing bath Ca^{2+} with Sr^{2+}, which enters motor terminals and mitochondria but does not support mPTP opening. In wild-type mice stimulation-induced Ψ_m depolarizations resembling those in symptomatic fALS mice could be elicited following prolonged exposure to diamide (200 μM), which produces an oxidative stress, but could not be elicited simply by increasing Ca^{2+} influx with 3,4-diaminopyridine (0.1 mM), which prolongs the action potential.

Discussion and conclusions: These results suggest that the increased Ψ_m depolarizations evoked by repetitive stimulation in motor terminals of symptomatic mutant SOD1 mice have at least 2 components: a component not affected by CsA (also present in presymptomatic mice), and a CsA-sensitive component. Thus as disease progresses from presymptomatic to symptomatic stages, the already reduced ability of mitochondria to maintain Ψ_m during stimulation-induced Ca^{2}+ entry is further compromised by increased activation of the mPTP. These transient mPTP openings may arise when Ca^{2}+ influx is combined with damage produced by oxidative stress. The large, cumulating Ψ_m depolarizations are likely to disrupt multiple aspects of mitochondrial function, including ATP production and sequestration of Ca^{2}+ loads, and may thereby contribute to motor terminal degeneration in fALS mice. Our results suggest that agents that reduce oxidative stress and/or mPTP opening might help preserve motor terminals. Supported by ALS Assn, MDA and NIH/NINDS.

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SESSION 11B RESPIRATORY AND NUTRITIONAL MANAGEMENT

C90 PREDICTING ENERGY NEEDS IN ALS: RESULTS OF THE ALS NUTRITION/NIPPV STUDY

KASARSKIS E[1,2], MENDIONDO M[1], KRYSCIO R[1], TANDAN R[3], POTTER C[3], MATTHEWS D[3], MITSUMOTO H[4], SIMMONS Z[5], BROMBERG M[1], WELLS S[1], HEALEY M[1], THE ALS NUTRITION/NIPPV STUDY GROUP[1]

[1]*University of Kentucky, Lexington, KY, United States,* [2]*VA Medical Center, Lexington, KY, United States,* [3]*University of Vermont, Burlington,VT, United States,* [4]*Columbia University, New York City, NY, United States,* [5]*Pennsylvania State University, Hershey, PA, United States,* [6]*University of Utah, Salt Lake City, UT, United States*

E-mail address for correspondence: ejkas@uky.edu

Keywords: energy expenditure, predictive equations, nutrition

Background: PEG placement in ALS is indicated when the metabolic (energy, calorie) needs of the body cannot be met via the oral route. The equations currently used in dietetic practice do not accurately predict the energy needs of an individual ALS patient at various stages of their illness. Hence published guidelines emphasize indirect indices of nutritional status such as weight loss, prolonged meal times, and dysphagia rather than the direct measurement of energy balance, ie, Energy Intake matching Total Daily Energy Expenditure (TDEE).

Objective: The goal of this study is to develop ALS-specific equations to predict the energy (calorie) requirements for ALS patients during disease progression.

Methods: TDEE was determined using the 'gold standard' Doubly Labeled Water method every 16 weeks over a 48 week period. We measured other factors that might influence TDEE including food intake, body composition (DXA, BIS), resting metabolic rate, physical activity, and ALS clinimetric scales. ALS subjects were entered into two groups (≥80% and 50-79% of predicted FVC) and were further stratified within each group according to their ambulatory status into subgroups based on walking ability using the ALSFRSr. Thus TDEE was determined as subjects progressed through varying combinations of ambulatory and respiratory impairment. Modeling TDEE was accomplished using linear mixed model procedures.

Results: TDEE and the other factors were available for 249 independent determinations in 80 subjects. The mean number of visits was 3.2±1.2 with at least two sequential measurements in 82.5%. Subjects had a mean age of 59.0±11.7 years, limb-onset presentation in 72.5% (n = 58), bulbar onset in 26.3% (n = 21), and generalized onset in 1.3% (n = 1). The mean sitting FVC in Group 1 was 89.2±7.2% of predicted and in Group 2, 63.9±9.5%. At baseline, the mean ALSFRSr score was 36.1±5.8 and the BMI was 27.1±5.7 kg/m^2 and 27.1±4.0 kg/m^2 in females and males, respectively. The mean change in the ALSFRSr was −1.09±0.07 points/month.

Discussion and conclusions: Our final equations will be presented. We developed a 'research' equation to predict TDEE with the most important factors being food intake, physical activity, and body composition. A second set of 'practical' equations were developed by modifying the Harris-Benedict, Mifflin-St. Joer, and other equations for ALS. The results of this study will allow us to provide equations to predict the energy requirements of ALS patients as their disease progresses. The study was funded by the National Institute of Neurological Disorders and Stroke (NINDS) grant 1 RO1 NS045087, the ALS Hope Foundation, and the Cynthia Shaw Crispen Endowment.

C91 NON-INVASIVE VENTILATION IN ALS: A 10-YEAR POPULATION-BASED STUDY IN ITALY

CHIÒ A[1], CALVO A[1], MOGLIA C[1], SELLITTI L[1], BALMA M[1], MAZZINI L[2], MORA G[3]

[1]*ALS Center, Department of Neuroscience, University of Torino, Torino, Italy,* [2]*ALS Center, Department of Neurology, Universrity of Eastern Piedmont, Novara, Italy,* [3]*ALS Center, Fondazione Salvatore Maugeri, IRCCS, Scientific Institute of Milano, Milano, Italy*

E-mail address for correspondence: achio@usa.net

Keywords: non-invasive ventilation, population-based study, outcome

Background: Recent guidelines (1,2) state that non-invasive ventilation (NIV) is the treatment of choice for respiratory failure (Class I evidence) in ALS. However, no data are available about the use of NIV in ALS patients and its impact on survival in a population-based setting.

Aim: To evaluate clinical characteristics and outcome of ALS patients who underwent NIV, using data from a prospective epidemiological register.

Methods: The Piemonte and Valle d'Aosta Register for ALS (PARALS) is a prospective epidemiological register established in 1995 collecting all ALS incident cases in two Italian regions. Data on NIV are prospectively collected using a standard form.

Results: Among the 1260 patients incident in the 1995-2004 period (mean annual crude incidence rate, 2.90/100,000 population), 243 (19.3%) underwent NIV. The number of NIV significantly increased from 88 (14.2%) in the period 1995-1999, to 161 (25.1%) in the period 2000-2004 (P =0.0001). The probability to undergo NIV was significantly higher for patients followed by a tertiary ALS centre (197; 32.8%) than those attending general neurological departments (52; 7.9%) (P =0.0001). Patients with spinal onset, younger than 69 and male had a significantly higher probability to undergo NIV.
The overall median survival time after NIV was 270 days. It was longer for patients followed by ALS tertiary centres (305 days vs. 150 days; P=0.0001). No difference in survival was found between bulbar and spinal patients, whereas age strongly influenced NIV outcome (median survival time: ≤49 years, 380 days; 50-69 years, 245 days ≥70 years, 210 days; P =0.0002).

Conclusions: The use of NIV is rapidly increasing in this population-based series, but mostly among patients attending tertiary ALS centers. Median survival time in our population is similar to that reported in clinical series and in controlled trials and is mainly related to patients' age. Similarly to a

previous survey on tracheostomy in the same population (3), younger age and male gender are strong predictors of the attitude to undergo NIV.

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C92 RISKS OF RESPIRATORY FAILURE, EMERGENCY HOSPITALIZATIONS AND UNPLANNED OUTCOMES OF ALS/MND PATIENTS: A 20 YEAR STUDY

CAZZOLLI P[1], BROOKS B[2], LEWARSKI J[3], MCKIM D[4], CHATBURN R[5]

[1]*ALS Care Project, Canton, OH, United States,* [2]*Carolinas Medical Center Department of Neurology, Charlotte, NC, United States,* [3]*Invacare Corporation, Elyria, OH, United States,* [4]*Ottawa Rehabilitation Centre, Ottawa, ON, Canada,* [5]*Cleveland Clinic Foundation, Cleveland, OH, United States*

E-mail address for correspondence: PCazzolliRN@aol.com

Keywords: respiratory failure, noninvasive ventilation, emergency hospitalizations

Background: ALS/MND results in respiratory failure, unless breathing support is used. Noninvasive positive pressure ventilation (NPPV) is the treatment of choice, but severe bulbar impairment limits its use. Inability to tolerate NPPV may occur, as oral secretions increase. ALS/MND patients may not have timely pulmonary monitoring when respiratory impairment advances. Signs when to initiate or use NPPV may be overlooked. Unexpected acute respiratory failure (ARF) may cause panic, resulting in emergency hospitalizations, early mortality or unintended tracheostomy positive pressure ventilation (TPPV).

Objectives: To identify the risks contributing to unexpected respiratory failure, emergency hospitalizations, failed use of NPPV and commencement of TPPV in patients with or without prior NPPV use.

Methods: In this observational study, 166 ALS/MND patients who had emergency ARF during use of mechanical ventilation (MV) were followed prospectively. An Oral Secretion Scale (OSS) was used to determine the level of oral secretions (Score 4 = normal; Score 3=minimal; Score 2 = moderate; Score 1 = substantial; Score 0 = profound) of the patients when they began NPPV, died or transitioned to TPPV.

Results: In 81/166 (49%) MV patients, NPPV was initiated. 14/81 (17%) attempted NPPV and died. 13/81 (16%) started TPPV, after NPPV failed. 17/27 failed NPPV initiation with OSS scores of 0-1. 16/81 (20%) began emergency NPPV following a 'good' pulmonary report. 54/81 (67%) continued NPPV use, survived 1 to 86.5 months. 21/54 (39%) began NPPV with emergency hospitalization. 12/21 (57%) were ambulatory. 14/21 (67%) had OSS scores of 4. At start of NPPV, 6/21 (29%) were nonresponsive, but NPPV reversed symptoms. 23 (43%) of 54 NPPV users had emergency hospitalizations prior to death. 6/23 (26%) patients, who had OSS score of 4, experienced unexpected ARF when off NPPV. 3/23 (13%) with OSS score of 4, who tolerated NPPV, had failed use of NPPV, when hospice gave morphine/oxygen. 14/23 (61%) with excessive oral secretions (OSS scores of 0 or 1) no longer tolerated NPPV. In contrast, 118 patients began emergency TPPV. 104 /118 (89%) did not plan TPPV ahead. 14/118 (12%) began planned TPPV during ARF. 50/118 (42%) were ambulatory. 34/118 (29%) had early respiratory onset. 10/118 (12%) had ARF post-PEG placement. 20/118 (6%) previously used NPPV. 10/20 (50%) of previous NPPV users had OSS scores 0-1 when NPPV became intolerable. 9/20 (45%) with OSS score of 4 had ARF when off NPPV.

Conclusions: OSS score of 1 signaled intolerance of NPPV and need for hospice or planned TPPV. Risks for ARF, failed use of NPPV, emergency hospitalizations, early mortality and unplanned tracheostomy: excessive oral secretions, intolerance and inadequate use of NPPV, unawareness of pending ARF, overexertion, walking, respiratory onset, lack of pulmonary monitoring, use of CPAP, post-PEG tube placement and use of morphine/oxygen in NPPV tolerant patients.

C93 SLEEP CHARACTERISTICS AND THEIR PREDICTIVE VALUE IN ALS

PATEL S[1], PANKRATZ N[3], IBER C[1,2], TIRYAKI E[1,2]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Hennepin County Medical Center, Minneapolis, MN, United States,* [3]*Indiana University, Indianapolis, IN, United States*

E-mail address for correspondence: etiryaki@gmail.com

Keywords: sleep, polysomnography, NIV titration

Background: Changes of sleep study characteristics in ALS have been previously described in small cohorts. While use of noninvasive ventilation (NIV) has been shown to extend survival, patient tolerance of NIV may vary. Little is known about sleep study characteristics and their predictive value for survival and respiratory complications.

Objective: The goal of this retrospective study was to describe clinical and sleep study characteristics in ALS patients and examine their predictive value for patient tolerance of NIV, respiratory complications and survival.

Methods: Consecutive patients with probable or definite ALS from a single center with polysomnography (PSG) from 2006 to 2010 were included. Standard PSG data and clinical characteristics were collected at multiple time points, including time from symptom onset to first respiratory complication (medical attention for dyspnea, aspiration or pneumonia).

Results: Fifty-six patients (52% male, mean age at onset: 57.5±3.1 years (range 30-82), 27% bulbar onset, mean BMI: 24.3±5.7) were studied. Median FVC at time of PSG was 47% of predicted. Sleep study characteristics were as follows: median respiratory rate during sleep was high at 16.3 (normal ≤14), median respiratory disturbance index (RDI) was 10.4, median apnea-hypopnea index (AHI) was 2.3, median respiratory event-related arousal (RERA) index was 4.6, median sleep efficiency was 61.8%, REM sleep was 6.9% (normal is >15%), nocturnal hypoxia (SpO2<88% >5 min) was 19.6% and percent of sleep related hypoventilation was 30.4%. Rate of disease progression was typical (median loss of 0.91 total ALSFRS points per month, median disease duration 2.9 years). Respiratory complications occurred in 16 individuals (29%).
Increased respiratory events (RERA and RDI) were significantly associated with occurrence of respiratory complication ($P < 0.05$). Each point of increase of these indices was associated with a 7% increase in risk of having a respiratory complication. Increased RERA and RDI and presence of

sleep-related hypoventilation trended toward lower survival 6-months after first respiratory complication. Treatment recommendation with supplemental O_2 based on PSG significantly reduced 6-month survival after first respiratory complication (P = 0.01).
Suboptimal treatment with NIV was associated with a trend toward lower 6-month survival after first respiratory complication (P = 0.07). Of those patients who did not tolerate NIV, 70% had upper extremity onset. These patients were four times more likely to not tolerate NIV compared to LE or bulbar onset patients (P = 0.07).

Discussion and conclusions: Altered sleep characteristics indicative of respiratory distress with nocturnal tachypnea and poor sleep efficiency are observed in this population. Increasing frequency of respiratory events during PSG is associated with increased risk of respiratory complications. Inability to optimally titrate NIV appears to be linked to poor clinical outcome. As patients with upper extremity weakness are less likely to tolerate NIV, development of strategies to improve NIV tolerance is important for clinical management.

C94 THE ROLE OF AIRWAY CLEARANCE VERSUS HYPOVENTILATION AND RESPIRATORY MUSCLE FATIGUE IN ALS

TIRYAKI E[1,2], PATEL S[1], PANKRATZ N[3], IBER C[1, 2]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Hennepin County Medical Center, Minneapolis, MN, United States,* [3]*Indiana University, Indianapolis, IN, United States*

E-mail address for correspondence: etiryaki@gmail.com

Keywords: airway clearance, hypoventilation, polysomnography

Background: Respiratory muscle involvement is a major predictor of survival in amyotrophic lateral sclerosis (ALS). Polysomnography (PSG) can be used to determine presence of hypoventilation and respiratory muscle fatigue and aid in titration of non-invasive ventilation (NIV).

Objective: To determine if impaired airway clearance is a stronger predictor of survival than hypoventilation or respiratory muscle fatigue.

Methods: Consecutive patients with probable or definite ALS studied with PSG from 2006 to 2010 were identified by chart review. Alteration in diet (dysphagia 1-3 or NPO as determined by formal speech evaluation) and drop in bulbar ALSFRS-R subscores for speech, salivation and swallowing at the time of PSG were used as proxies for impairment of airway clearance. Data regarding hypoventilation and respiratory muscle fatigue were captured at PSG, including CO_2 retention during sleep (delta TCM>10), increased respiratory rate during sleep (>14/min), hypoxia (SpO_2 <88% >5 min) and recommendation to initiate treatment. ALSFRS subscores for dyspnea and orthopnea were also used to determine respiratory involvement. Primary outcomes were time from onset to death and time to first respiratory complication (medical attention for dyspnea, aspiration or pneumonia).

Results: Data from 56 patients were analyzed. Rate of disease progression was typical (median loss of 0.91 total ALSFRS points/month, median disease duration 2.9 years). Rate of decline of forced vital capacity (FVC) accounted for 20% (P =0.02) of the variance in survival. A bulbar ALSFRS subscore ≤ 6 (of 12) at time of PSG explained 10% of variation in disease duration while a respiratory subscore ≤ 4 (of 8) explained 0%. Modification of diet explained 10% of the variation in disease duration (P = 0.08). Each respiratory PSG parameter explained less than 4%.
Respiratory complications occurred in 16 individuals (29%) and median time to first respiratory complication was 3.2 years. The rate of decline of FVC accounted for 24% of the variation in time to first respiratory complication (P = 0.09). Modification in diet explained 19%, while respiratory subscores accounted for 3%. None of the examined respiratory PSG parameters, including the recommendation to treat with NIV, accounted for more than 6%.

Discussion and conclusions: While most tests failed to reach statistical significance due to the small sample size, our preliminary data suggest that the risk for respiratory complications of ALS and survival are more strongly predicted by impairment in airway clearance than by hypoventilation or respiratory muscle fatigue. While important for titration of NIV, PSG does not capture airway clearance and as such does not fully assess variables that affect disease progression. Additional focus on aggressive management of airway clearance may need to be considered for optimal clinical management of ALS. The large Northeast ALS Consortium database will be analyzed to further test our hypothesis; these results will be presented at the meeting.

C95 USING LUNG VOLUME RECRUITMENT THERAPY TO IMPROVE SWALLOWING AND AIRWAY PROTECTION FOR INDIVIDUALS WITH ALS

CLEARY S[1], MISIASZEK J[1], WHEELER S[2], KALRA S[1], JOHNSTON W[1]

[1]*University of Alberta, Edmonton, Canada,* [2]*Misericordia Community Hospital, Edmonton, Canada*

E-mail address for correspondence: stuart.cleary@ualberta.ca

Keywords: rehabilitation, respiration, swallowing

Background: Lung volume recruitment (LVR) is a manual breath stacking technique used to help patients with ALS cough with sufficient force to clear pulmonary secretions. LVR has been associated with positive treatment outcomes; however much of the evidence for its use is anecdotal and many questions remain about the nature of the treatment effect.

Objectives: The purpose of this study was to answer the following research questions: 1) What is the immediate intensity and duration of the LVR treatment effect on standard tests of pulmonary function? 2) Does LVR improve volitional airway clearance behaviours (ie, hawking, throat clearing, unassisted coughing and forced expiration)? 3) What is the effect of LVR on a specific compensatory swallowing technique (the supraglottic swallow maneuver)? 4) What are participants' perspectives on the effects of LVR on their respiration and secretion management?

Methods: Participants were 29 individuals with ALS and were currently doing LVR. The mean forced vital capacity of the group was 58% of predicted norms and the median ALSFRS-R score was 28/48. The study involved a within-subjects, repeated measures cross-over research design. Measurements were collected on each participant under two conditions: LVR treatment and no treatment. The order of these conditions was counterbalanced to control for order effects. Assessments of pulmonary function, coughing, airway clearance and swallowing were conducted at three time points: baseline,

immediately after the treatment, and 30 minutes after the treatment was provided.

Results: The results of this study were as follows: 1) LVR had a significant positive effect on forced vital capacity immediately post-treatment but did not have a facilitative effect on sniff nasal pressure at any time point; 2) LVR had an significant positive effect on peak cough flow (PCF) during unassisted coughing, hawking, throat clearing, and forced expiration immediately after treatment and 30 minutes post treatment; 3) LVR had a significant positive effect on PCF measured during the supraglottic swallow maneuver, immediately after and 30 minutes post-treatment; 4) The majority of participants expressed satisfaction with LVR, indicating that they tolerated the treatment well, and that they perceived positive effects on cough strength and the ability to clear the airway.

Discussion: Findings have important clinical implications for the symptom management of individuals with ALS. The current rationale for doing LVR is to clear secretions, but if scheduled prior to meals this treatment may provide enhanced airway protection and clearance over the length of a typical meal. In addition, the results help inform theoretical models of mechanisms of the LVR treatment effect. LVR is hypothesized to increase lung volumes and improve coughing effectiveness by recruiting collapsed lung segments, improving compliance and range of motion of the lung and chest wall and eliminating flow limitations within the airway.

C96 FINAL ANALYSIS OF PILOT TRIAL OF DIAPHRAGM PACING (DP) IN ALS/MND WITH LONG TERM FOLLOW-UP: NO SAFETY CONCERNS AND DP POSITIVELY AFFECTS DIAPHRAGM RESPIRATION

ONDERS R, KATIRJI B, SCHILZ R, ELMO M, KAPLAN C

University Hospitals Case Medical Center, Cleveland, Ohio, United States

E-mail address for correspondence: raymond.onders@uhhospitals.org

Keywords: respiratory failure, diaphragm pacing, diaphragm function

Background: Respiratory insufficiency is the major cause of mortality in patients with ALS. Ventilators, although life-saving, are only used by a very small percentage of patients. Alternate therapy to prevent or manage respiratory muscle decline in ALS is needed. The motor point diaphragm pacing (DP) pilot trial formed the basis for the multi-center pivotal trial. This reports the final outcome for the initial patients implanted.

Objective: Analyze safety, utility and long term use of the DP system from the initial prospective pilot FDA single site study.

Methods: Patients underwent outpatient laparoscopic diaphragm motor point mapping with electrode implantations. Stimulus/output characteristics of each electrode were determined and diaphragm conditioning initiated. Patients conditioned their diaphragms with 5 daily stimulation sessions of 30 minutes each but were allowed to increase usage. Each patient had three extensive lead-in assessments that were continued post implantation of the DPS system and included pulmonary function tests, fluoroscopic evaluation of diaphragm movement, ultrasound analysis of diaphragm thickness, and quality of life tests.

Results: From 3/05 to 3/07, 16 patients were implanted with no peri-operative or unanticipated device related adverse events. Seven had feeding tubes placements with or after DP. Average age was 50 (range 32-70) with 13 males. Patients were a median 35 months from symptoms at enrolment with a mean ALSFRr of 27 and FVC of 60% predicted at surgery. No patients stopped DP use because of pain, discomfort or failure of implanted electrodes. Four patients are still surviving with 3 on tracheostomy mechanical ventilation. There have been a total of 352 implant-months of follow-up with an average of 22 months per patient post implant. One patient was excluded due to an abdominal co-morbidity of colon cancer diagnosed post implant. Median tracheostomy-free survival was 18.6 months from DP implant, 43.4 months from diagnosis and 56 months from initial onset. In all patients, fluoroscopically observed diaphragm excursion was greater with diaphragm stimulation than under maximal voluntary effort. DP significantly increased muscle thickness when assessed with ultrasound (P-value 0.02). Paired FVC rate of decline (treatment - lead in) improved with DP $1.47 \pm 2.18\%$ per month ($P=0.03$). NIV was never used by 7 of the 16 patients and 50% of patients used DP to overcome central sleep apnea. The cause of death or tracheostomy mechanical ventilation include respiratory failure(5 or 33% of all events), traumatic fall (1) aspiration (3), peri-operatively from a cervical spinal fixation (1), urosepsis (1), colon cancer (1) and three patients had to turn off DP to allow death during final hospice care.

Conclusion: Long term analysis of the DP system in ALS showed no safety issues. The pilot data suggests that DP can positively influence diaphragm physiology, respiratory function and survival in ALS/MND.

SESSION 12 JOINT CLOSING SESSION

C97 UNDERSTANDING THE SYNDROMIC NATURE OF MOTOR NEURON DISEASE

LEIGH PN

King's College London, London, United Kingdom

E-mail address for correspondence: n.leigh@iop.kcl.ac.uk

Keywords: phenotypes, biology, 'omics'

Clinical practice requires a conceptual paradigm that is pragmatic above all else. The diagnosis must be correct (most of the time), and the advice given on prognosis and treatment must (more or less) be accurate. The clinicopathological revolution of Cruveilher, Charcot and their contemporaries and followers established the conceptual paradigm under which we, in the era of 'Omics', still labour.

The 'Charcot worldview' is certainly serviceable in the clinic. However, just as classical notions of the constellations and galaxies tell us little about astrophysics, so the identification of phenotype with theory may be more fanciful than helpful. How do we escape from this intellectual corral? First, we might try to agree on what phenotypes are important to study, although this is tautological! By defining the phenotypes, we perpetuate their (apparent) importance. Nonetheless, we can certainly improve our understanding of the (imperfect) descriptive tools in use (eg, the labels 'typical', bulbar onset', flail arm, flail leg, PMA, and so on).

However, I am not convinced that this 19th century solution is useful any longer. The labels we use are most likely misleading surrogates for the complex genetic and epigenetic factors that contribute to the development and manifestation of ALS. The key step therefore is to use basic indicators of biology (for example, age of onset, race, gender, genetic risk) and rate of spread ('disease intensity') and survival, to understand groupings that have undeniable biological significance. This is where unbiased approaches to analysis of phenotype are essential. For example, if we use a traditional phenotypic classification to study prognosis (ie, biology), we find interesting differences between 'typical ALS' and the flail arm and flail leg syndromes in terms of prognosis and sex distribution. However, taking an 'unbiased' approach – latent cluster analysis, provides a very different set of groups, with two factors accounting for most of the differences between groups.

An ALS study population can be divided up in all kinds of ways, depending on the prejudices of the investigators. To move beyond our prejudices (if such is possible) we may find that subdividing our population samples simply on the a few robust biological factors (eg, age of onset; groupings based on latent cluster analyses or similar approaches; short or long survival) is the most informative approach. It remains to be seen how biomarkers of disease fit into this paradigm, but traditional clinical phenotyping – bedside, pathology, has now outlived its usefulness for ALS research. A new consensus is needed.

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THEME 1 THERAPEUTIC STRATEGIES

P1 ANALYSIS OF MOTOR SKILL ACQUISITION AMONG CHILDREN WITH TYPE I SPINAL MUSCULAR ATROPHY SUBMITTED TO MEDICATION WITH VALPROIC ACID

CONCEIÇÃO E[1], SILVA T[2], UMBERTINA R[1], MARIA JOAQUINA D[1]

[1]*Universidade de São Paulo, São Paulo , Brazil,* [2]*Universidade Federal de São Paulo, São Paulo, Brazil*

E-mail address for correspondence: erikacgouveia@gmail.com

Keywords: Type I Spinal Muscular Atrophy, valproic acid, motor acquisition

Background: Spinal Muscular Atrophy (SMA) is the most frequent of the neuromuscular diseases responsible for hypotonia (floppy infant syndrome) caused by the degeneration of cells in the anterior horn of the spinal cord. Symptoms consist of progressive, symmetrical motor deficit of the affected limbs and the condition sometimes affects the musculature innervated by the cranial nerves.Valproic acid has proven effective in avoiding the deletion of exon 7, which is thought to prolong the survival of neurons. Based on this new treatment possibility, a physical-functional assessment protocol was developed for monitoring this group of patients.

Objective: The aim of the present study was to analyze and describe possible motor acquisitions among patients with SMA-I submitted to a medication with valproic acid and assess whether observational analysis using filming is a resource of easy application and adequate reproducibility as a form of evaluating and following up such patients.

Methods: Between November 2005 and December 2007, five infants with a confirmed diagnosis of SMA-I between four and 18 months of age were evaluated. The inclusion criteria were SMA-1 (definition based on the European Neuromuscular Centre Workshop), difficulty breathing on the first evaluation and no need for assisted ventilation. Data collection was carried out following a previously structured protocol involving the determination of motor acquisition using filming with the patient in dorsal decubitus.

Results: Fifteen patients with a confirmed diagnosis of SMA-I submitted to treatment with valproic acid between November 2005 and December 2007 were evaluated. Three patients died; six became dependent on mechanical ventilation during the evaluation period, with the evaluations and medication subsequently interrupted; and one patient did not undergo the proposed physiotherapy because the parents were not available to participate. At the end of the data collection, only patients who underwent the five evaluation sessions were considered eligible; thus, the results analyzed were on only five patients, all of whom were Caucasian. The results demonstrated that none of the children exhibited a worsening of the motor skills, which would be expected in the natural progression of the disease.

Conclusion: Based on the results of the present study, valproic acid may be considered a medication that assists in the maintenance of motor skills as well as new acquisitions, as none of the children who participated in the study exhibited any worsening in motor ability, which would be expected in the natural progression of the disease.

P2 UNPROVED CELLULAR TREATMENTS FOR ALS PATIENTS: AN OBSERVATIONAL STUDY

GAMEZ J

ALS Unit, Neurology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain, Autonomous University of Barcelona, Barcelona, Spain

E-mail address for correspondence: 12784jgc@comb.es

Keywords: stem cells, ensheathing cells, cellular therapy

Background: No curative therapy for ALS is yet available, and the drug treatment used has an almost imperceptible effect on the disease's clinical course. Cellular therapies have recently become a promising strategic approach in the treatment of neurodegenerative diseases, as they may replace dysfunctional or dying neurons. Cell transplantation may provide other benefits, including neuronal growth factors for the host cells, stimulation of axonal growth or glial function, secretion of neurotransmitters deficient in the host, differentiation into oligodendrocytes and myelination of host axons and differentiation into neurons. Many patients are using the Internet to seek these innovative procedures at clinics offering a variety of cellular treatments for ALS before their effectiveness and safety is confirmed. The increasing number of clinics offering 'cure-all' therapies for financial gain, taking advantage of patients' understandable desperation to find a cure, has prompted considerable 'stem cell tourism'.

Objectives: To analyze the effectiveness of cellular therapy (TX) in a series of 12 Spanish ALS patients undergoing cytotherapy in clinics found on the Internet on their own account.

Methods: Twelve ALS patients with a mean age of 48.6 years old (SD 12.8) received cytotherapy 26.9 months (SD 15.8) after clinical onset. ALSFRS-R and FVC at TX were 32.3 (SD 6.8) and 63.4% (SD 15.3) respectively. TX involved transplants of olfactory ensheathing cells in three patients, and autologous mesenchymal stem cells in the remainder.

Results: One patient died 33 months post-TX, after surviving for 49 months. Five required mechanical non-invasive home ventilation 7.4 months post-TX. Two required invasive ventilation (13.0 months post-TX). Five patients needed

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gastrostomy feeding (23.3 months post-TX). Survival between clinical onset and the study end date was 50.0 months (SD 17.2). No significant adverse events or changes in the decline of FVC and ALSFRS-R compared to the disease's natural history were observed.

Discussion: The ever-increasing availability of stem cells in many countries, often offered by "for profit" clinics, together with straightforward laws of supply and demand, raises ethical implications. These clinics provide therapies that are not proven which may cause serious psychological and economic distress to the patients concerned, and endanger the legitimate progress made by scientists in the stem cells field.

Conclusions: Our observations and those of other groups suggest that cell therapy in ALS is not yet sufficiently effective in curing or halting the disease, and its presumed ability to slow down the disease's progress is not yet proven. Cytotherapy cannot yet be considered a curative treatment for ALS. Patients' associations and experts treating the disease must strongly dissuade their patients from undertaking the worrying new type of medical tourism arising from their desperate search for effective treatment.

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P3 STRENGTHS AND WEAKNESS OF THE HISTORICAL CONTROL DESIGN FOR SCREENING TRIALS IN ALS: AN ANALYSIS OF THE WALS MULTICENTER LITHIUM TRIAL

KATZ J, MILLER R, MOORE D

California Pacific Med Center, San Francisco, CA, United States

E-mail address for correspondence: jonskatz@gmail.com

Keywords: clinical trials, historical controls, clinical progression

Background: The WALS group recently completed a multicenter study of Lithium. Because of circumstances related to the positive Lithium study from Italy, we used Minocycline historical placebo controls (MP) and compared them with actively treated Lithium patients. Our work was supplemented by additional data from 748 placebos from 7 earlier trials. Our methodology maximized efficiency, acknowledged Lithium was available by prescription, and avoided ethical conflicts in case the agent turned out effective. The year-long trial enrolled 109 patients, and concluded Lithium was not a candidate for further development.
Now, we wish to focus specifically on our methodology to discuss implications for future trials. Since our trial is complete, we have a unique opportunity to study biases and efficiency tradeoffs, especially since other trials were performed simultaneously.

Methods: We tested four rules regarding the validity of historically controlled trials: 1) There should be no drift in the primary endpoint over time. We compared the change in ALSFRS-R of placebo groups from different epochs; 2) There should be no differences in types of patients that enrolled in the separate trials. This might relate to the open design, or to variations in timing or screening strategies. Here, we compare baseline demographics between this study and our historic control group; 3) No factors should lead to variations in drop outs that could secondarily bias conclusions. We compare dropout rates in our cohort and the historical control group; 4) Simultaneous trials should reach similar conclusions. This is a way to check for other potential biases such as a lack of blinding. We discuss two other Lithium trials, one using randomization and blinding, done concurrently to ours.

Results: 1) There is no change in the 6-month FRS slopes between 1997 and 2007, with mean FRS declines of near 1.0/mo. for 7 trials; 2) There were no differences in baseline features (Lithium, MP): FVC (94.7, 94.3); FRS-R (37.1, 37.9); months since disease onset (18.5, 16.8); age, gender, bulbar involvement or riluzole use; 3) Dropouts due to death or other causes were 19% for MP and were 18% for Lithium; 4) Two concurrent trials reported Lithium was ineffective, one with concurrent controls. All found trends suggesting treated subjects fared worse than controls and clearly refuted the original positive study. All found similar trends in secondary outcome measures (FVC slopes).

Conclusions: There were no clear differences in types of patients enrolled or rates of progression between distinct trials. This lends credibility to the historical-control design for screening new agents. As long as these trials are performed under conditions that parallel earlier work, the risk of introducing bias seems small and given the savings in cost and acceptance, the trade-off may be of benefit to the field.

P4 LESSONS LEARNED FROM LITHIUM TRIALS IN ALS

MILLER RG, KATZ J, MOORE D

California Pacific Medical Center, San Francisco, CA, United States

E-mail address for correspondence: millerrx@sutterhealth.org

Keywords: clinical trials, trial design, therapy

Background: In 2008, Fornai *et al*, reported in PNAS that lithium dramatically slowed disease progress in both a mouse model and a small trial with 16 subjects with ALS (1). Supported by scientific background related to autophagy, the trial generated an unprecedented reaction with many patients taking lithium and a groundswell of activity to test the agent further. Eventually, over 700 patients were enrolled in eight clinical trials organized in five countries, using a variety of methods to re-test the result.

These events give us a unique opportunity to inspect different approaches for screening compounds and to examine the most effective ways to utilize resources.

Methods and objective: We evaluated designs (inclusion criteria, sample size, treatment duration, outcome measures, controls) and findings (efficacy and safety) of these trials with two specific goals: 1) to determine which were most efficient as screening trials; and 2) to learn how to develop a more optimal response for new agents.

Results: There were four open-label case series (2–5) (three US, one French), one dose ranging study (6) (Italy) and three placebo-controlled trials (7–9) (US/Canada, ongoing UK, ongoing Italian). The trials differed in sample size from 27 to 220.
All but the English (8) and Italian (9) trials are complete and all except the initial small trial (1) have reported negative results. None were terminated based on reports of an earlier trial being negative.

All four case series reported frequent adverse events with one stopped for safety (2–5). Two compared outcomes to historical values and concluded that lithium was not effective (4,6). One placebo controlled trial using a novel 'time-to-event' endpoint was terminated at the first interim analysis due to futility (7). The three completed controlled trials found no therapeutic benefit from lithium (4,5,7). Two randomized controlled trials are ongoing (8,9).

Conclusions: Despite differences, these trials have thus far, consistently refuted the initial Italian study. This suggests a variety of designs might be practical, especially when the goal is to screen for phase III or test the possibility of a very large beneficial effect. The findings point to the need for a more efficient overall approach to screening since the current atmosphere sets the stage for committing too many resources to a single agent. These lithium trials lend a considerable opportunity to ask why any given evidence leads to such a large number of trials and how to organize research across borders.

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P5 AUTOMATED DATA CHECKS PROCESSES: IMPROVING DATA QUALITY IN CLINICAL TRIALS AND BIOMARKER STUDIES

CHAN M, SINANI E, SHERMAN A, YU H, CUDKOWICZ M

Massachusetts General Hospital, Charlestown, MA, United States

E-mail address for correspondence: mchan5@partners.org

Keywords: data management, neurology clinical trials unit, EDC

Introduction: The Neurology Clinical Trials Unit at Massachusetts General Hospital serves as a data management center for multi-site clinical trials and biomarker studies. It utilizes PharmaENGINE™ software platform for data capture and data management. Clinical Data Management is the process of ensuring that data collected during the course of a clinical trial are accurate, complete, logical and consistent. The amount of data accumulated during a clinical trial may be significant and depends on a trial's duration, the number of subjects, number of sites and number of procedures performed. Manual data validation in most cases is not a feasible alternative as it is an inefficient process that most likely results in invalid data and, as a result, incorrect analysis and results interpretation. An automated data checks functionality built into PharmaENGINE™ is used in both data entry and data verification to detect incorrect or incomplete data.

Methods: The PharmaENGINE™ system has been designed to check for data errors and data consistency through two methods: automated queries at the point of entry and logic checks.

- **Automated Queries** detects data discrepancies within a form such as missing fields, out-of-window visits, out-of-range values, and unknown values with no comments associated with them.
- **Logic checks** functionality that is built into the system allows detection of data discrepancies within a form or across multiple forms and visits. The module allows entering SQL based scripts that will check the data, specify the field for which an automatic query will be created if the script returns positive results, and provide the query message.

A query is automatically created in a field or a form that has a discrepancy. The trial personnel is able to check for any outstanding queries.

Results: In early 2010, the Logic Checks functionality was introduced for the Clinical Trial of Ceftriaxone in subjects with amyotrophic lateral sclerosis. This multi-center trial has 58 participating sites and a target enrollment of 600 subjects. There were a total of 2216 queries opened in the month of February alone; 45% of these queries were created by the automated queries and logic checks combined.

Conclusions: Utilizing Automated Queries and Logic Checks is a must for any data management effort in a multi-site clinical trial. The alternative is time-consuming and costly, not only in human resources utilization, but also from a high risk of missing important data entry errors that may lead to invalid results and their interpretations. Running Logic Checks takes minutes versus weeks and months for the manual alternative. Hence, these tools should be viewed as an important asset in service of data management professionals.

P6 EFFECTS OF TREATMENT WITH DEXPRAMIPEXOLE ON ALSFRS-R SUB-DOMAIN SCORES AND CROSS-VALIDATION OF CHANGE IN SUB-DOMAIN SCORES WITH PREVIOUSLY PUBLISHED DATA

INGERSOLL E[1], CUDKOWICZ M[2], MITSUMOTO H[3], MATHER J[1], ARCHIBALD D[1], GRIBKOFF V[1], BOZIK M[1]

[1]*Knopp Neurosciences Inc., Pittsburgh, PA, United States,* [2]*Massachusetts General Hospital, Boston, MA, United States,* [3]*Columbia University, New York, NY, United States*

E-mail address for correspondence: eingersoll@knoppneurosciences.com

Keywords: dexpramipexole, clinical trial, ALSFRS-R

Background and objectives: Dexpramipexole (KNS-760704; [6R]-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazolediamine), a compound that may be cytoprotective by ameliorating mitochondrial dysfunction, is being developed to treat ALS. The objective of this study was to evaluate changes in the 4 sub-domains of the ALS Functional Rating Scale, Revised (ALSFRS-R) in Study KNS-760704-CL201 (CL201) for treatment effects and, within the placebo group, for concordance with previously published patterns of sub-domain dynamics (1). A treatment effect in an ALS clinical trial would be most likely observed within sub-domains that exhibit the greatest change in the reference group over the treatment period.

Methods: Results of safety and clinical effects in study CL201 have been reported previously (2). This was a randomized,

multicenter, double-blind, placebo-controlled study in ALS subjects. A total of 102 subjects were randomized to receive 50, 150, or 300 mg/day dexpramipexole or placebo for 12 weeks. Clinical effects were, in part, measured by the ALSFRS-R, which has 4 sub-domains (fine motor, gross motor, bulbar, and respiratory), each assessed according to 3 questions directed to specific activities of daily living. Cedarbaum and colleagues previously showed that the greatest decline in any of the ALSFRS-R sub-domains at 3 months was in the fine motor sub-domain (estimated as −0.9 absolute/40% of total score change), followed, in order, by the gross motor (−0.65/29%), bulbar (−0.4/18%) and respiratory (0.3/13%) sub-domains. In study CL201, the patterns of change in the ALSFRS-R sub-domains were compared for treatment differences, and the placebo group was compared with those reported by Cedarbaum *et al* over a comparable time period.

Results: The patterns of change in ALSFRS-R sub-domain scores observed by Cedarbaum *et al* over a 3 month period were replicated in the placebo cohort of this study, with the greatest mean decline observed in the fine motor sub-domain (−1.4/38% of total score change), followed by gross motor (−0.9/24%), bulbar (−0.8/22%), and respiratory (−0.6/16%) sub-domains. In the 300 mg dexpramipexole group, mean changes were −0.6 (fine), −0.9 (gross), −0.3 (bulbar), and −0.5 (respiratory). The largest magnitude nominal treatment effect was in the fine motor domain.

Discussion and conclusions: The concordance between the placebo cohort in this study and the population studied by Cedarbaum *et al* provides external validation for the ALSFRS-R as a measure of functional decline in a randomized clinical trial. The greater magnitude of decline observed in CL201 may be a function of differences in inclusion criteria, but the pattern and relative percentages of decline were similar across the sub-domains. The sub-domain with the greatest decline over 12 weeks in the placebo group was fine motor, in which domain the largest magnitude treatment effect with 300 mg dexpramipexole was also seen.

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P7 MECHANISMS OF NEUROPROTECTION BY HSP90 INHIBITORS IN MODELS OF FALS

DURHAM H[1], CHA J[1], TRADEWELL M[1], ZINKIE S[1], JAFFER Z[2], CHEN R[2], RUBINSTEIN A[2]

[1]*McGill University, Montreal, QC, Canada,* [2]*NexGenix Pharmaceuticals, New York, NY, United States*

E-mail address for correspondence: heather.durham@mcgill.ca

Keywords: Hsp90 inhibitor, stress response, culture model

Background: Many therapeutic strategies for ALS are designed to reduce cellular stress or upregulate endogenous stress responses to compensate for the high vulnerability of motor neurons to the disease. Relative to astrocytes, motor neurons have a high threshold for transactivation of stress response genes, including heat shock and phase II antioxidant genes. Evidence that these properties compromise maintenance of protein quality control in ALS includes: chaperoning activity is decreased in lumbar spinal cord of presymptomatic $SOD1^{G93A}$ mice; specific proteasomal enzyme activities are reduced in lumbar cord of P45+ $SOD1^{G93A}$ mice; chymotrypsin-like activity is decreased in sporadic ALS spinal cord, but not in cerebellum (unpublished data).

Objectives: The Hsp90 inhibitors, geldanamycin and NXD 30001 (a novel radicicol-based Hsp90 inhibitor with improved *in vivo* pharmacodynamic properties from NexGenix Pharmaceuticals), were highly neuroprotective in a primary culture model of fALS1, preventing aggregation of mutant SOD1 into inclusions and prolonging viability of motor neurons. The objectives of this study are to determine: 1) If Hsp90 inhibitors prevent other manifestations of mutant SOD1 toxicity, including calcium dysregulation and mitochondrial abnormalities; 2) If Hsp90 inhibitors exert neuroprotection through reducing cellular burden of mutant protein either through upregulation of HSPs or by disrupting association of Hsp90 with mutant SOD1 or other client proteins.

Methods: Mutant or wildtype SOD1 (+/− eGFP tag) are expressed in motor neurons of dissociated spinal cord-DRG cultures by intranuclear microinjection of plasmid construct. Microfluorometric techniques are used to measure levels of Ca^{2+} in mitochondria, ER and cytoplasm; mitochondrial shape, fission/fusion and axonal transport; formation of inclusions, and viability in individual motor neurons. To estimate levels of plasmid-derived eGFP-tagged protein in motor neurons and background cells, microscopic images are captured using a Hamamatsu cooled CCD camera (500 msec exposures). MetaFluor software (Universal Imaging) is used to calculate pixel density of fluorescence in defined regions.

Results and conclusion: NXD30001, as geldanamycin, induced HSPs, ie Hsp70 and Hsp40, but not Hsp90. Also upregulated was clusterin, a chaperone expressed in motor neurons and astrocytes and well known for its secretion and chaperoning function in the extracellular space. NXD30001 reduced accumulation of SOD1-$eGFP^{G93A}$ in motor neurons following gene transfer. 40 μM NXD30001 significantly reduced accumulation of SOD1-$eGFP^{G93A}$ on both day 1 and 2 following microinjection, but had minimal effect on accumulation of SOD1-$eGFP^{WT}$. Significant reduction of SOD1-$eGFP^{G93A}$ was measured on day 2, but not on day 1 following treatment with 5 μM NXD30001, demonstrating a dose-response effect. These results provide evidence that Hsp90 inhibitors are protective at least in part by reducing levels of mutant protein. Studies on mitochondrial manifestations of toxicity are in progress and NXD30001 is under further testing and therapeutic development for ALS.

P8 ADMINISTRATION OF RECOMBINANT METALLOTHIONEIN-I STARTING AT ONSET PROLONGS SURVIVAL IN A MOUSE MODEL OF MUTANT SOD1-LINKED FAMILIAL ALS

TOKUDA E[1,2], ONO S-I[1,3]

[1]*Nihon University, Funabashi, Japan,* [2]*Umea University, Umea, Sweden,* [3]*Akiru Municipal Clinical Center, Tokyo, Japan*

E-mail address for correspondence: eiichi.tokuda@gmail.com

Keywords: metallothionein, non-neuronal cell, transgenic mouse

Background: Mutations in the gene of copper/zinc superoxide dismutase (SOD1) are the most common known cause of amyotrophic lateral sclerosis (ALS). Non-neuronal cells, including glial cells, influence motor neuron survival in

ALS and are a potential target to prevent motor neuron degeneration.
Metallothionein-I (Mt-I), a metal binding protein, is primarily located within glial cells. When neurons are injured, Mt-I is released by glial cells toward the injured neurons where it acts to promote neuronal survival and regeneration. Overexpression of Mt-I prolongs survival of SOD1G93A mice (1), whereas, absence of Mt-I shortens survival (2,3).

Despite the fact that Mt-I plays a critical role in protecting motor neurons, it is not known whether administration of Mt-I ameliorates mutant SOD1 toxicity.

Objectives: The aim of the present study was to elucidate the effects of intraperitoneal administration of recombinant Mt-I (rMt-I) starting at disease onset on the disease course in $SOD1^{G93A}$ mice.

Methods: rMt-I was purchased from Alexa Biochemicals. Since rMt-I contains 5~7% zinc ions, we used two types of control groups: a zinc sulfate ($ZnSO_4$) group and a phosphate buffered-saline (PBS) group. $SOD1^{G93A}$ mice (B6SJL, high copy number) were assigned to receive a daily intraperitoneal administration of rMt-I (0.5 mg/kg: n = 24), $ZnSO_4$ (0.025 mg/kg: n = 24), or PBS (n = 24). Treatment was started at 13 weeks of age, when the $SOD1^{G93A}$ began to exhibit ALS-like symptoms.
The clinical onset of the disease was evaluated by examining mice to identify shaking of the limbs when suspended in the air by the tail. The end-point was defined as inability of a mouse to right itself within 30 s after being pushed onto its side.
For analysis of the survival of the motor neurons, lumbar sections of the spinal cord were immunostained using anti-NeuN antibody. The number of NeuN-positive motor neurons in the ventral horn was counted.

Results: Treatment of recombinant Mt-I prolonged the survival of the mice by 17% (rMt-I: 146±2.8, $ZnSO_4$: 124±3.2, PBS: 122±1.9 days). The progression of the disease was slowed by 78% (rMt-I: 52±7.1, $ZnSO_4$: 29±2.6, PBS: 27±2.0 days).
At 17 weeks of age, SOD1G93A mice had only 50% surviving motor neurons, whereas 77% remained in mice treated with rMt-I.

Conclusions: rMt-I might be a potential therapeutic target for familial ALS patients with SOD1 mutations.

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P9 INTRAMUSCULAR ADMINISTRATION OF METALLOTHIONEIN-IIA IMPROVES SURVIVAL OF G93A SOD1 MOUSE

FOSTER S, LEWIS K, WEST A, CHUNG R, CHUAH MI

University of Tasmania, Hobart, Tasmania, Australia

E-mail address for correspondence: Inn.Chuah@utas.edu.au

Keywords: metallothionein, G93A SOD1 mouse, intramuscular injection

Background: Mutations to superoxide dismutase 1 (SOD1) enzyme have been linked to familial Amyotrophic Lateral Sclerosis. These mutations typically cause a toxic gain-of-function resulting in tyrosine nitration and protein aggregation and excitotoxicity (1). Mutant SOD1 also has a higher affinity to aggregate when it is zinc deficient (2). The neuroprotective protein metallotheionein-IIA (MT-IIA) is involved in metal homeostasis, particularly that of zinc, and can act as an antioxidant (3).

Objectives: (i) To determine whether MT-IIA can delay neurodegenerative decline and improve survival in the G93A SOD1 transgenic mouse; (ii) To investigate a possible route through which MT-IIA can access the CNS.

Methods: At 10 weeks of age, MT-IIA or a saline control was injected intramuscularly into the left hindlimb of litter pairs comprising a wild type and a mutant G93A SOD1 mouse twice a week until the mice reached endstage (loss of 20% maximum body weight). At each injection time point, weight and disease symptoms (muscle wastage, hindlimb mobility and tremors) were assessed. In addition, mice deficient in MT (MTKO) were used to investigate whether there is any difference in tissue access when MT-IIA is administered intramuscularly or intraperitoneally.

Results: Symptom analysis showed that from 147 days of age, the MT-IIA treated group showed significantly less severe symptoms compared to the control group. There was also improved survival rate in the MT-IIA treated group compared to the control group, beginning at 145 days of age. Survival of the control and MT-IIA treatment group was 15% and 40% respectively at 150 days of age. Histological analysis of MTKO showed that MT-IIA was present in blood vessels as soon as 15 minutes after intramuscular injection. Intraperitoneal injection resulted in detection of MT-IIA in the proximal convoluted tubules of the kidney 15 minutes later, suggesting that the MT-IIA may have been taken up rapidly in the vascular circulation and formed a component of the glomerular filtrate.

Discussion and conclusions: These results suggest that MT-IIA can delay neurodegenerative decline and prolong survival in the G93A SOD1 mouse. The evidence suggests that both intramuscular and intraperitoneal injection can rapidly deliver MT-IIA to the blood circulation. Recent studies show that the blood brain barrier in the G93A SOD1 transgenic mouse is altered (4). An important question is whether the MT-IIA in blood circulation can access the CNS of the G93A SOD1 mouse readily and is therefore able to act on the neurons.

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P10 NO BENEFIT FROM CHRONIC DIMEBOLIN DOSING IN A SIBLING-MATCHED, GENDER BALANCED, INVESTIGATOR-BLINDED TRIAL USING A STANDARD MOUSE MODEL OF FAMILIAL ALS

VIEIRA F, MORENO A, KIDD J, THOMPSON K, DHILLON H, GILL A

ALS TDI, Cambridge, MA, United States

E-mail address for correspondence: jlincecum@als.net

Keywords: SOD1-G93A, dimebolin, therapy

Background: Dimebolin is a non-selective anti-histamine drug approved in Russia for treatment of allergy. The drug has been shown to be protective in a beta-amyloid fragment Abeta 25-35 cerebellar granule cell model that may mimic some neurodegenerative aspects of Alzheimer's Disease and has been demonstrated as neuroprotective in glutamate-induced apoptosis in striatal neuronal cultures derived from a Huntington's disease mouse model. Dimebolin preserves learning and memory after chronic administration in a preclinical AF64A cholinergic-lesion in rats. Dimebolin has not been tested for efficacy in any preclinical models of motor neuron degeneration.

Objectives: The aim of the current study was to test chronic dimebolin dosing for efficacy in a sibling-matched, gender balanced, investigator-blinded trial using the high-copy (average 23 copies) SOD1G93A mouse (n= 30 per group).

Methods: Pharmacokinetic parameters in high-copy SOD1G93A mice of dimebolin were determined after single 30 mg/kg intraperitoneal injection of dimebolin dihydrochloride in 1X phosphate buffered saline. The parameters were used to establish a dosing regimen for chronic delivery of dimebolin in SOD1G93A mice which could result in constant exposure of drug to the spinal cord. In the survival efficacy study, treatment commenced when mice were approximately 55 days old. Dimebolin-treated mice received a single loading dose of 1.75 mg/kg on the first day of treatment and were subsequently implanted with Alzet osmotic mini-pumps which delivered 384 µg of dimebolin free-base per day to each mouse in 6 µL of 1X PBS. Exhausted pumps were removed and replaced after 30 days for the duration of the study as necessary. Neurological disease severity score and body weight were determined daily during the dosing period. Age at onset of definitive disease and survival duration were recorded. Summary measures from individual body weight changes and neurological score progression, age at disease onset, and age at death were compared using Kaplan-Meier and Cox proportional hazards analysis.

Results and conclusions: Rigorous survival study design that includes sibling matching, gender balancing, investigator blinding, and transgene copy number verification for each experimental subject minimized the likelihood of attaining a false positive therapeutic effect in this standard animal model of familial ALS. Results from this study do not support taking dimebolin into human clinical trials for ALS.

P11 TREATMENT WITH THE UPA INHIBITOR WX-340 DELAYS ONSET OF CLINICAL SYMPTOMS IN G93A ALS MICE

HENSLER M[1], LORENZL S[1], SCHMALIX W[1]

[1]*Department of Neurology, LMU, Munich, Germany,* [2]*Wilex AG, Company, Munich, Germany*

E-mail address for correspondence: stefan.lorenzl@med.uni-muenchen.de

Keywords: uPA, uPA inhibitor WX-340, G93A mice

Purpose: We have recently shown that treatment with the uPA inhibitor WX-340 (Wilex AG, Germany) significantly prolonged survival of G93A mice. The objective of this study was to investigate whether delayed start of treatment with WX-340 alters disease progression.

Methods: 150 transgenic mice were used. Mice were grouped as follows: vehicle group d60 (start of treatment), WX-340 10 mg/kg d30, WX-340 1 mg/kg d60, WX-340 10 mg/kg d60, Riluzole 30 mg/kg/day d60, and WX-340 10 mg/kg + Riluzole 30 mg/kg/day d60 (administered in drinking water). Clinical scoring and body weight was performed once-a-week, open field test measurement clinical onset of disease (score = 4) and survival data were recorded.

Results: There were no differences in the body weight between the treatment groups. Number of rearings was significantly decreased on age week 15 in WX-340 1 mg/kg d60 and WX-340 10 mg/kg d60 plus Riluzole treatment groups compared to vehicle group. Clinical score was significantly improved on age week 11 in WX-340 1 mg/kg d60 treatment group compared to vehicle group. The WX-340 10 mg/kg d60 group had a significantly delayed onset of clinical symptoms. There were no significant differences in the survival between the treatment groups.

Conclusions: WX-340 administered at dose of 10 mg/kg starting at the age of 60 days significantly delayed the onset of clinical symptoms. Chronic treatment with 1 or 10 mg/kg of WX-340 at the age of 60 days has no significant effects on survival, body weight, or open field activity.

P12 COPPER BIS(THIOSEMICARBAZONATO) COMPLEX ADMINISTRATION REDUCES TDP-43 PATHOLOGY, DELAYS DISEASE ONSET AND EXTENDS SURVIVAL IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

SOON C[1,2], DONNELLY P[4], TURNER B[3], CROUCH P[1,2], SHERRATT N[1,2], TAN J-L[1], LIM N[1,2], LAM L[2], BICA L[1], LIM S[4], HICKEY J[4], CULVENOR J[1], DUCE J[1,2], MASTERS C[1,2], WHITE A[1,2], BARNHAM K[1,2], LI Q-X[1,2]

[1]*The University of Melbourne, Department of Pathology, Parkville, Australia,* [2]*The Mental Health Research Institute, Parkville, Australia,* [3]*Florey Neuroscience Institutes, Parkville, Australia,* [4]*The University of Melbourne, School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, Parkville, Australia*

E-mail address for correspondence: q.li@unimelb.edu.au

Keywords: SOD1G93A, CuII(atsm), TDP-43

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by

deterioration of motor neurons with no effective treatment. Diacetylbis(N(4) methylthiosemicarbazonato)copper(II) (CuII(atsm)) is a coordination complex that crosses the blood-brain barrier and has antioxidant properties.

Objective: This study examined the effects of systemic CuII(atsm) treatment on disease onset and progression in the SOD1G93A mouse model of ALS.

Methods: TgSOD1G93A mice in C57B6 background with delayed phenotype due to low copy of transgene were characterized previously (1) and administered CuII(atsm) orally at 30 mg/kg at both pre-symptomatic and symptomatic age. Clinical assessment and motor function tests including rotarod and stride length, as well as biochemical and histological analysis were performed.

Results: CuII(atsm) pre-symptomatic treatment led to 70% increase in survival after motor impairment onset and 14% increase in overall lifespan. Importantly, treatment beginning at symptom onset also increased survival after motor impairment onset by 59%, and prolonged overall lifespan by 10%. These effects were accompanied by rescue of motor neurons, attenuation of oxidative damage, suppression of astrocyte and microglial activation and prevention of pathologic up-regulation of metalloproteinase 9 in spinal cords, compared to vehicle-treated control mice. Furthermore, CuII(atsm) treatment reduced abnormal phosphorylation and cleavage of TDP-43.

Discussion and conclusion: This reduction in pathological changes may contribute to the neuroprotective effect of CuII(atsm) in this mouse model. The study also provides a link between disease progression and TDP-43 pathology, which can be pharmacologically modulated. This study indicates that CuII(atsm) may be a promising therapeutic drug candidate for ALS.

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P13 ACTIVATION OF THE TRANSCRIPTION FACTORS CREB AND NRF2: MECHANISM OF ACTION STUDIES TO DEFINE THE POSITIVE THERAPEUTIC OUTCOMES FOR CU(ATSM) IN ALS MODEL MICE

CROUCH P[1,2], PATTERSON B[1], LIM S[1], SOON C[1,2], MASTERS C[1,2], LI Q-X[1,2], DONNELLY P[1], BARNHAM K[1,2], WHITE A[1,2]

[1]*University of Melbourne, Melbourne, Victoria, Australia,* [2]*Mental Health Research Institute of Victoria, Melbourne, Victoria, Australia*

E-mail address for correspondence: pjcrouch@unimelb.edu.au

Keywords: therapeutic, mechanism of action, transcription factor

Background: The paucity of effective therapeutic treatments for ALS is highly disproportional to the devastating impact ALS has on the people affected. The development and testing of new and more effective therapeutic strategies is therefore an important research goal. Cu^{II}(atsm) is a metal-ligand complex that crosses the blood-brain barrier after oral administration. We have shown that treating a $SOD1^{G93A}$ mouse model of ALS with Cu^{II}(atsm) improves locomotor function and delays paralysis when administered pre- or post-symptom onset.

Objective: This study examined Cu^{II}(atsm) at a cellular level in order to elucidate the mechanisms through which Cu^{II}(atsm) improves locomotor function in ALS model mice.

Methods: Neuronal- and glial-like cells were grown in culture and treated with Cu^{II}(atsm) or its vehicle control. Treatments were performed under normal growing conditions, or under conditions to induce a specific cellular stress (hypoxia, glucose deprivation, glutathione depletion etc).

Results: Treating neuronal-like SH-SY5Y cells with Cu^{II}(atsm) under normal growing conditions showed that although the compound is taken up by these cells it is relatively inert under normal conditions. Stress conditions such as hypoxia and glutathione depletion however induced Cu^{II}(atsm)-mediated responses including activating phosphorylation of the signalling kinase Akt, inhibitory phosphorylation of GSK3β, and activating phosphorylation of the transcription factor CREB. In contrast to neuronal like SH-SY5Y cells, we found that glial-like U87-MG cells responded to Cu^{II}(atsm) in the absence of any specific cellular stress. U87-MG responses to Cu^{II}(atsm) included activation of Akt and up-regulation of Nrf2, a transcription factor that regulates levels of cellular antioxidants.

Discussion and conclusions: These mechanistic studies indicate the therapeutic outcomes for Cu^{II}(atsm) in ALS model mice involve the activation of transcription factors with the potential to increase synaptic activity (CREB activation in neuronal cells) and protect against oxidative insult (Nrf2 activation in glial cells). Our on-going work aims to define the mechanism of action for Cu^{II}(atsm) in greater detail in order to better understand its potential as a therapy for ALS and to define the relevance of CREB and Nrf2 activation as valid therapeutic targets.

P14 NEUROPROTECTION OF RAPAMYCIN AND COMBINED TREATMENT OF RAPAMYCIN AND GSK-3 INHIBITOR IN AN ALS MOUSE MODEL

AHN S-W, YOUN Y-C, PARK K-Y, SHIN H-W, KWON O-S, JANG J-W, KIM K-E

Chung-Ang University Hospital, Seoul, Republic of Korea

E-mail address for correspondence: icandr@hanmail.net

Keywords: autophagy, rapamycin, GSK-3 inhibitor

Background: In previous studies of neurodegenerative disorders, autophagy is the major route for lysosomal degradation of misfolded protein aggregates and oxidative cell components. Thus, the study of the autophagy pathway and its therapeutic effect is important in ALS. In addition, it is well known that GSK-3 plays significant roles in intracellular apoptotic pathways, and the suppression of GSK-3 activity could be one of the potential pathogenic mechanisms of ALS.

Objective: Given that apoptosis and autophagy play a central role in motor neuron degeneration and can contribute to neuronal death through distinctive routes in ALS, we hypothesize that up-regulation of autophagy would prolong the survival of motor neurons and suppress the disease progression in ALS. Moreover, the concurrent administration with both down-regulation of apoptosis and up-regulation of autophagy would probably have additive effects on neuronal survival and motor function.

Methods: A total of 24 transgenic mice harboring the human G93A mutated SOD1 gene and 6 wild type mice were used following confirmation of their genotype. The 24 transgenic mice were equally divided into 4 groups; transgenic control, GSK-3 inhibitor treatment, rapamycin treatment and concurrent administration group. The clinical status, rotarod test and survival of the ALS mice model was evaluated beginning 60 days after birth. Cumulative probabilities of symptom onset, rotarod failure, and endpoint were analyzed with the Kaplan-Meier survival analysis.

Results: The onset of symptoms was significantly delayed in both GSK-3 inhibitor treatment group and rapamycin treatment group, and non-significantly delayed in concurrent administration group relative to the control group. In addition, motor activity using rotarod test was significantly improved in both rapamycin treatment group and concurrent administration group in incipient stage, and non-significantly improved in GSK-3 inhibitor treatment group relative to the control group. The time of rotarod failure and endpoint were non-significantly delayed in GSK-3 inhibitor group relative to the control group. However, unexpectedly, the time of rotarod failure and end point were shortened in both rapamycin treatment group and concurrent administration group relative to the control group, although the results were not statistically significant.

Conclusion: The present study suggest that: GSK-3 inhibitor has a neuroprotective effect which could delay disease progression in the ALS mouse model consistent with previous reports; treatment with rapamycin in incipient stage of ALS mouse model delayed motor function deficit and onset of symptoms; excessive dose or prolonged treatment of rapamycin exacerbated the disease progression in the ALS mouse model. Thus we suggest that an appropriate dose of rapamycin would be a novel promising therapeutic strategy in ALS, although this is a preliminary study with a few limitations.

P15 CHRONIC TREATMENT WITH THE MACROLIDE IMMUNOSUPPRESSANT RAPAMYCIN IN A SIBLING-MATCHED, GENDER BALANCED, INVESTIGATOR-BLINDED TRIAL ACCELERATES NEUROLOGICAL DISEASE PROGRESSION IN A STANDARD MOUSE MODEL OF FAMILIAL ALS

VIEIRA F, MORENO A, KIDD J, THOMPSON K, GILL A

ALS Therapy Development Institute, Cambridge, MA, United States

E-mail address for correspondence: jlincecum@als.net

Keywords: G93A-SOD1, rapamycin, immunomodulation

Background: Rapamycin is an immunosuppressive macrolide antibiotic that binds to the cytosolic protein FKBP12. The rapamycin-FKBP12 complex inhibits the mammalian target of rapamycin (mTOR) pathway by directly binding the mTOR Complex1 (mTORC1). Immunomodulatory treatment with an anti-Cd40lg monoclonal antibody delays onset of paresis and extends survival in the high copy G93A-SOD1 mouse model, but its mechanism of action remains unclear. Like anti-Cd40lg monoclonal antibodies, macrolide immunosuppressants have been used in preclinical models of organ transplant to improve likelihood of engraftment. It is possible that macrolide immunosuppressants like rapamycin and FK506 could have downstream mechanistic overlap with anti-Cd40lg therapy that would be efficaceous in the G93A-SOD1 mouse model of ALS.

Objectives: The aim of the current study was to test chronic rapamycin dosing for efficacy in a sibling-matched, gender balanced, investigator-blinded trial using the high-copy (average 23 copies) G93A -SOD1 mouse (n=30 per group).

Methods: Pharmacokinetic parameters in high-copy G93A-SOD1 mice of rapamycin were determined after single 5 mg/kg intraperitoneal injection of rapamycin in 1% DMSO in 1x phosphate buffered saline confirming peripheral and central nervous system exposure of the drug in mice. A sibling-matched, gender balanced, investigator-blinded efficacy study was initiated in G93A-SOD1 mice. Treatment commenced when mice were approximately 55 days old. Treatment cohort mice received daily injections of 1 mg/kg rapamycin in 1% DMSO in phosphate buffered saline. Neurological disease severity score and body weight were determined daily during the dosing period. Age at onset of definitive disease and survival duration were recorded. Summary measures from individual body weight changes and neurological score progression, age at disease onset, and age at death were compared using Kaplan-Meier and Cox proportional hazards analysis.

Results and conclusions: In this initial test of early (55 days of age) immunomodulation using daily 1 mg/kg IP rapamycin treatment, body weight was less well maintained, neurological disease showed an earlier onset, and neurological symptoms progressed more rapidly than in vehicle control animals. There was no significant change in survival compared to vehicle treated control mice.

P16 GRANULOCYTE COLONY STIMULATING FACTOR REDUCES INFLAMMATION IN A MOUSE MODEL OF ALS

POLLARI E[1], KANNINEN K[1], GINIATULLINA R[1], AHTONIEMI T[2], MALM T[1], GINIATULLIN R[1], KOISTINAHO J[1], MAGGA J[1]

[1]*A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland,* [2]*Medeia Therapeutics Ltd, Kuopio, Finland*

E-mail address for correspondence: eveliina.pollari@uef.fi

Keywords: G-CSF, G93A-SOD1 mice, inflammation

Background: Amyotrophic lateral sclerosis (ALS) is a lethal motoneuron affecting neurodegenerative disease and currently has no known cure. We propose that granylocyte colony stimulating factor (G-CSF), a neuroprotective agent, is a promising drug candidate for treatment of ALS. G-CSF is clinically used to mobilize stem cells from bone marrow and to treat neutropenia. It promotes angiogenesis and neurogenesis, has anti-apoptotic and anti-inflammatory effects and is neuroprotective in models of several acute and chronic diseases.

Objectives: Our aim was to assess G-CSF mediated protection to the central nervous system (CNS) and to the inflammatory status in mouse model of ALS.

Methods: Transgenic mice overexpressing mutant Cu, Zn superoxide dismutase, G93A-SOD1, received weekly subcutaneous injections of G-CSF starting at pre-symptomatic stage. At symptomatic stage and at end-stage disease we analyzed an array of inflammatory markers and determined reactive oxygen species (ROS) in CNS. Immunohistochemical

antibody stainings were performed to evaluate gliosis and neuronal survival in lumbar spinal cord (SC) at end-stage disease. Additionally, disease progression and survival were monitored. Neuroprotective effect of G-CSF was confirmed in primary SC cultures.

Results: G-CSF prolonged survival and slowed down the disease progression from the onset to death in transgenic G93A-SOD1 mice. Production of proinflammatory cytokines was reduced in G-CSF treated transgenic mice in comparison to untreated transgenic mice both at symptomatic and end-stage disease. In addition, G-CSF treatment reduced gliosis and promoted neuronal survival in the ventral horn of lumbar SC and diminished ROS levels in the CNS at the end-stage. Furthermore, in primary neuronal cultures we were able to verify neuroprotective properties of G-CSF.

Discussion and conclusions: Our data reveal new anti-inflammatory properties of G-CSF in a mouse model of ALS. We were able to demonstrate that G-CSF prolongs survival of G93A-SOD1 mice and protects neurons in culture. These results validate beneficial properties of G-CSF as a neuroprotective factor for the treatment of ALS.

P17 THE PLEOTROPHIC EFFECTS OF IGF-I ON HUMAN SPINAL CORD NEURAL PROGENITOR CELLS

LUNN S[1], PACUT C[1], BACKUS C[1], HONG Y[1], JOHE K[3], HEFFERAN M[2], MARSALA M[2], FELDMAN E[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*University of California San Diego, La Jolla, CA, United States,* [3]*Neuralstem, Inc., Rockville, MD, United States*

E-mail address for correspondence: slunn@umich.edu

Keywords: stem cell, IGF-I, therapeutic

Background: Amyotrophic lateral sclerosis (ALS) is a lethal neurological disorder that leads to progressive degeneration and loss of motor neurons (MN). Neural progenitor cells derived from the spinal cord have the potential to differentiate into various cell types, including both neurons and glia, within the disease microenvironment. Combining growth factors with Schwann cells, glia, and stem cells delivers both cellular and neurotrophic support. Insulin-like growth factor-I (IGF-I) is a growth factor with neuroprotective properties highly considered for treatment of ALS. The combination of human spinal cord stem cells (HSSC) with IGF-I treatment may provide a systems approach to the treatment of ALS as well as other motor neuropathies

Objectives: Our hypothesis is that IGF-I in combination with HSSC will enhance HSSC integration into the host tissue and offer greater neuroprotection in neurodegenerative diseases. Our goal is to understand the role of IGF-I in stem cell biology and how IGF-I may interact with stem cells to increase their neuroprotective effects.

Methods: HSSC were prepared and provided by Neuralstem, Inc. Briefly, cells were prepared from a cervical–upper thoracic region of spinal cord tissue obtained from a single 8-week human fetus after an elective abortion. The fetal tissue was donated by the mother in a manner fully compliant with the guidelines of NIH and FDA and approved by an outside independent review board. The direct effects of IGF-I on the differentiation of HSSC were assessed either by western blotting or immunocytochemistry. Cell death was assessed using TUNEL.

Results: Our findings demonstrate that IGF-I is produced early in HSSC differentiation. Direct treatment with IGF-I does not change the expression profile of HSSC as they are differentiating, suggesting IGF-I does not affect lineage selection, but rather enhances neural development and neurite outgrowth. Signaling via AKT, but not MAPK mediates IGF-I-stimulated neurite outgrowth. HSSC are more resistant to glutamate excitotoxicity than mature MNs. As such, HSSC may be less susceptible to pathogenic factors while they mature; however, IGF-I remains a potent neuroprotective factor for excitotoxic stress in HSSC. Finally, IGF-I does not promote proliferation of HSSC.

Discussion and conclusions: We show that IGF-I increases the proportion of HSSC producing neurites and increases neurite length. Furthermore we show that IGF-I mediated AKT signaling is required for neurite extension. IGF-I does not change the proliferation profile of differentiating HSSC, or the terminal fate decisions. We go on to show that IGF-I protects HSSC against glutamate-induced excitotoxicity. Our data support the idea that upregulation of IGF-I production in HSSC may offer additional therapeutic benefits when HSSC engineered to overexpress IGF-I are transplanted into the nervous system of animal models and humans with neurological disorders.

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THEME 2 *IN VIVO* EXPERIMENTAL MODELS

P18 EFFECT OF HUMAN MESENCHYMAL STEM CELLS INTRACARDIAC TRANSPLANTATION ON SOD1-G93A MICE

ZHANG C[1], ZHAO C-P[1], WANG Y-H[1], XIE Y-M[2], ZHOU C[1], LI W-Y[1]

[1]*Department of Neurology, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China,* [2]*Department of Neurology, University of North Carolina, Chapel Hill, North Carolina, United States*

E-mail address for correspondence: chengzhang100@hotmail.com

Keywords: human mesenchymal stem cells, SOD1-G93A mice, transplantation

Background: Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, neurodegenerative disease, currently without any effective therapy. Multiple advantages make mesenchymal stromal cells (MSC) a good candidate for cellular therapy of ALS. Usually, the MSCs were transplanted to SOD1-G93A mice by intravenous injection, but the lung arrests a lot of the BMSs. In order to overcome the problem, may we transplant the MSCs to SOD1-G93A mice by artery injection?

Objective: To study the changes of life span and pathology in SOD1-G93A mice after intracardiac transplantation of human mesenchymal stem cells (hMSCs).

Methods: hMSCs were isolated from bone marrow cells obtained from healthy donors and cultured. The purity and morphology were assessed by flow cytometry (FCM). hMSCs (3×10^6) resuspended in 0.2 ml DMEM was injected into the heart of 8 week-old SOD1-G93A mice. In non-transplantation control SOD1-G93A mice, only DMEM was injected. The mice were evaluated for signs of motor deficit with a 4-point scoring system previously described by Weydt *et al* (1). The age of onset and life span in mice were assessed. The pathological change including number of motor neurons was investigated by Nissl staining. Immunofluorescence staining with specific human nuclear antibody was used to confirm the transplant of hMSCs in mice.

Results: The onset of symptoms in untreated SOD1-G93A mice appeared at (156.56±3.60) days of age and the average life span was (188.32±3.51) days. hMSCs transplantation delayed the onset of ALS type symptoms about 16 days ($\chi^2=10.888$, $P=0.001$) and prolonged the life span about 14 days compared to the untreated SOD1-G93A littermates ((202.19±4.09) days *vs* (188.32±3.51) days, $\chi^2=3.917$, $P=0.04$). The loss of motor neurons in untreated mice was earlier and more severe than in hMSCs transplanted mice. At 20 weeks, the number of motor neurons in transplanted mice was significantly higher than those in untreated mice. Human specific nuclear antigen in brain and spinal cord was detected in transplanted SOD1-G93A mice.

Discussion and conclusions: hMSCs can be implanted for a long-term into the central nervous system by intracardiac transplantation and the transplantation can prolong life span, and delay the onset of the disease and motor neuron loss in SOD1-G93A mice.

Reference:

1. Weydt P, Hong SY, Kliot M, *et al*. Neuroreport 2003;14: 1051–4.

P19 FLUOXETINE TREATMENT HAS MODEST EFFECTS ON DISEASE PROGRESSION IN THE mSOD1G93A MOUSE MODEL OF ALS

SCHUSTER J, FU R, HECKMAN CJ

Northwestern University Feinberg School of Medicine, Chicago, IL, United States

E-mail address for correspondence: jennaschuster2010@u.northwestern.edu

Keywords: fluoxetine, serotonin, excitability

Background: Amyotrophic Lateral Sclerosis (ALS) is an adult onset neurodegenerative disease that results in the retraction of spinal motoneuron axon terminals from muscle fibers and motoneuron death. Since the discovery of inherited genetic mutations in some ALS patients and subsequent development of transgenic ALS mouse models, studies have shown that many factors contribute to disease onset and progression. Many of these factors can be aggravated directly or indirectly by increasing motoneuron excitability. Serotonergic inputs to spinal motoneurons are known to increase motoneuron excitability by increasing persistent inward currents (PICs) which amplify synaptic inputs. Fluoxetine (trade name Prozac), a selective serotonin reuptake inhibitor (SSRI) and a commonly prescribed antidepressant, may therefore have detrimental effects when given to ALS patients.

Objectives: The objective of this study was to determine if increasing synaptic serotonin levels negatively effects the progression of ALS by increasing motoneuron excitability.

Methods: In this study, fluoxetine was administered to the mutant superoxide dismutase G93A ($mSOD1^{G93A}$) mouse model of ALS. Fluoxetine was administered in the drinking water with target concentrations of either 5 or 10 μg/g/day. Groups were gender balanced. Treatment was started at postnatal day 70 (P70) near the onset of overt motor symptoms (ie appearance of tremor, mean 83±9) and continued until death. Water consumption, weight, rotorod performance, tremor severity (4 point scale), and end stage were monitored. Repeated measure ANOVAs were used for water consumption, weight, and rotorod performance over time with sex as a covariate. Bonferroni adjustments were made for multiple comparisons. Kaplan Meier log rank tests were used for time to tremor onset, the first decrease in rotorod performance, and end stage with the factor (drug concentration) being pooled for each stratum (sex).

Results: Fluoxetine treatment slightly increased tremor severity ($F=3.759$, $P=0.041$) and the decline in rotorod performance

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(3.774, P=0.041); although post hoc tests did not reveal any pair wise differences. The time to the first decrease in rotorod performance was also significantly different in female mice with performance decreasing sooner in the 10 μg/g/day (chi-squared=6.261, P=0.044) but not male mice (chi-squared=3.666, P=0.160). There were no main effects of drug treatment on water consumption, weight, tremor onset, or end stage.

Discussion: Fluoxetine given near symptom onset had modest effects on disease progression. This is encouraging given the frequent administration of SSRIs and other antidepressant medications to ALS patients. Ongoing studies will determine the effects of fluoxetine administered at earlier time points and will test the apparent increase in tremor severity with ventral root recordings in adult mice.

Conclusion: Fluoxetine, given at symptom onset, does not affect end stage in the SOD1G93A ALS mouse model. It may however increase the severity of tremors and, in females, accelerate the onset of motor impairment.

P20 HUMAN SOD1/G93A-EXPRESSING MICE PROVE INCREASED EXOCYTOTIC RELEASE OF GLUTAMATE

BONANNO G[1,2], MILANESE M[1], ONOFRI F[1], MUSAZZI L[3], TARDITO D[3], BONIFACINO T[1], MESSA M[4], GIRIBALDI F[1], BENFENATI F[1,4], POPOLI M[3]

[1]*Department of Experimental Medicine,* [2]*Center of Excellence for Biomedical Research; University of Genova, Genova, Italy,* [3]*Center of Neuropharmacology - Department of Pharmacological Sciences and Center of Excellence on Neurodegenerative Diseases, University of Milano, Milano, Italy,* [4]*Department of Neuroscience and Brain Technologies, The Italian Institute of Technology, Gevova, Italy*

E-mail address for correspondence: bonanno@pharmatox.unige.it

Keywords: SOD1/G93A mice, glutamate release, exocytosis

Selective vulnerability of motor-neurons in ALS has been in turn ascribed to protein misfolding, mitochondrial dysfunction, oxidative damage, insufficient growth factor signaling, inflammation and glutamate-mediated excitotoxicity. High glutamate levels have been reported in ALS patients and in animal models of the disease, and reduced glutamate transport, mainly due to astroglial GLT1 defects, was suggested as a cause. Due to the complex interplay of multiple mechanisms in the aetiology of ALS, defects of glutamate transport may not be the only reason for excitotoxicity-based neurodegeneration and other causes should be considered for the increased glutamate availability, including increase of glutamate release. We have previously found that the non-exocytotic glutamate release, due to activation of glycine and GABA transporters, heterologously sited on glutamate-releasing nerve terminals, is enhanced in the spinal cord of mice expressing human SOD1 carrying the G93A mutation (SOD1/G93A(+)), a widely used animal model of ALS. We extend here our investigation to the study of the modifications of the exocytotic release of glutamate, as a potential cause for hyper-glutamatergicity in ALS. The spontaneous outflow of endogenous glutamate and of the glutamate analogue [^{3}H]D-aspartate was more elevated in SOD1/G93A(+) mice, as compared to mice expressing wild type human SOD1 or to non-transgenic controls. Exposure to 15 mM KCl or 0.3 μM ionomycin provoked Ca^{2+}-dependent glutamate release that was dramatically increased in symptomatic transgenic mutated mice. Contrary to glutamate, the stimulus-evoked release of [^{3}H]GABA or [^{3}H]glycine in the spinal cord of SOD1/G93A(+) mice did not differ from controls, and the same was true for [^{3}H]D-aspartate release in the motor-cortex. The augmentation of basal and stimulated glutamate release was already present in pre-symptomatic mutant mice, suggesting it as a cause instead of a consequence of the progression of pathology. Further studies revealed the existence of increased resting and stimulated Ca^{2+} levels in nerve terminals from the SOD1/G93A(+) mouse spinal cord, accompanied by increased activation of Ca^{2+}/calmodulin-dependent kinase II and increased phosphorylation of synapsin I, a cascade of events leading to higher availability of synaptic vesicles for exocytosis. In line with these findings, release experiments suggested the involvement of the readily releasable pool of vesicles in the production of the abnormal glutamate release in SOD1/G93A(+) mice, as well as a greater capability of these vesicles to fuse upon stimulation.
To conclude, glutamate exocytosis is elevated in symptomatic and pre-symptomatic G93A mutant mice and changes in cytosolic Ca^{2+}, Ca^{2+}/calmodulin-dependent protein-kinase II auto-activation and synapsin I phosphorylation seem to be major causes of the augmented neurotransmitter release. The discovery of this excessive glutamate exocytosis may have translational perspectives. Presynaptic release inhibition is a common regulatory process of CNS transmission; already available or newly designed efficacious agents can be identified to selectively inhibit abnormal glutamate exocytosis in ALS.

P21 OVER-EXPRESSION OF GLUTAMATE CYSTEINE LIGASE CATALYTIC SUBUNIT IN THE LUMBAR SPINAL CORD OF SOD1-G93A TRANSGENIC MICE

GUO Y, LIU Y, LI Z, WANG Q, ZHANG K, HUANG J, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: GCLC, astrocyte, transgenic mice

Background: Glutamate cysteine ligase catalytic subunit (GCLC) regulates GSH synthesis and induction of GCLC is capable of enhancing the antioxidant capability of the cell. GCLC expression in the central nervous system of amyotrophic lateral sclerosis (ALS) SOD1-G93A transgenic mouse model has not been previously examined.

Objectives: To investigate whether GCLC expression is changed in the susceptible spinal cord as well as in the motor cortex.

Methods: Immunohistochemistry, Western blot and confocal microscopy were used to detect GCLC expression and its cellular location.

Results: The results showed that GCLC was up-regulated increasingly in the lumbar spinal cord of SOD1-G93A transgenic mice when the disease progressed. GCLC expression was located in neurons and did not show any co-localization with GFAP-positive astrocytes. Remnant interneurons in the lumbar anterior horn over-expressed GCLC and may contribute to the combat against motor neuron-destroying oxidative stress. In contrast, no alteration of GCLC expression was observed in the motor cortex of SOD1-G93A transgenic mice at any disease stages.

Discussion: The main discovery of this study was that over-expression of GCLC, the catalytic subunit of glutamate-cysteine

ligase, was examined in the lumbar spinal cord of SOD1-G93A transgenic mice and increased as the disease progressed. In contrast, GCLC over-expression was not found in the motor cortex of the transgenic mice. With the degeneration of motor neurons, interneurons in the lumbar anterior horn over-expressed GCLC, which might contribute to the antioxidant response. However, the dramatically proliferated astrocytes did not show any GCLC immunoreactivity. To the best of our knowledge, this is the first report about GCLC expression in the CNS tissues of SOD1-G93A transgenic mice.

Conclusions: It is suggested that GCLC up-regulation may represent a protective response against motor neuron degeneration in ALS.

P22 GENETIC BACKGROUND EFFECTS ON LIFESPAN OF SOD1 MOUSE MODELS OF ALS

SHER R, SOUNDARARARJAN P, COX G

The Jackson Laboratory, Bar Harbor, ME, United States

E-mail address for correspondence: roger.sher@jax.org

Keywords: modifier, genetic background, embryonic stem cells

Background: Dominant SOD1 mutations account for ~25% of familial forms of ALS (FALS), though heterogeneity in age of onset or symptom severity exist within families carrying the same SOD1 mutation, suggesting that modifier genes significantly impact the disease. Similarly, there is variation in onset and severity in hSOD1Tg mice on genetically heterogeneous backgrounds. For testing genetic modifiers of the disease, it is crucial to eliminate genetic heterogeneity in the animals to reduce phenotypic variability.

Objectives: The goals of our studies were to test the hypothesis that genetic modifiers can significantly affect the onset or progression of ALS symptoms in G93A mutant SOD1 transgenic mice, and to identify major QTL loci associated with longevity.

Methods: We developed a range of inbred strains containing the SOD1-G93A mutation with varying lifespans. One long-lived and one short-lived strain were used in reciprocal backcrosses for QTL analysis of modifier loci.

Results: We identified two inbred strains (ALR/LtJ, NOD/LtSz-Rag1^{tm1Mom}) that significantly accelerate disease, and three that significantly delay disease (C57BL/6J, DBA/2J and BALB/cByJ). Through reciprocal backcrosses between B6 (~161d) and ALR (~114d) lines we have mapped two major QTL on Chr 4 (LOD 4.76) and Chr 17 (LOD 11.99) that significantly modifies the lifespan of G93A SOD1 mice. The Chr 17 locus has also been identified at DUCOM through our collaborative research.
We crossed B6.SOD1-G93A with a B6.NOD-Chr17 congenic. Lifespan for SOD1 NOD/B6 mice (N=14) was 149.8±9.0 d, and for SOD1 NOD/NOD mice (N=14) was 136.4±7.0 d, a statistically significant difference between groups and from the B6.SOD1 line (all $P<0.001$). The decrease in lifespan is regulated in a dose-specific manner, with one NOD copy resulting in an ~7.1% lifespan reduction, and two NOD copies resulting in an ~15.4% reduction.

Discussion and conclusions: We have demonstrated that the genetic background of hSOD1-G93A transgenic mice significantly affects lifespan, and that a region of Chr 17 has a major dose-dependent effect on lifespan.
We are presently crossing the B6.NOD-Chr17 congenic to B6.SOD1-G85R and B6.SOD1-G37R lines to determine if this region has a general effect on SOD1-ALS lifespan. We are also crossing a NOD.B10-Chr17 congenic line to our NOD.SOD1-G93A line to determine if this increases lifespan in a short-lived strain. In addition, crosses of a TDP-43 mutant mouse to the B6.NOD-Chr17 congenic will determine if the Chr 17 QTL affects lifespan in other ALS models.
We have resequenced the Chr 17 region for mutations in gene(s) affecting lifespan. We are deriving embryonic stem cells from the G93A, G85R, and G37R SOD1 mice, along with the TDP-43 mouse, and are differentiating them into motor neurons. We will use these motor neuron cultures to test for effects of up- and down-regulation of genes of interest in our region.

P23 DIFFERENTIAL REGULATION OF THE GLUTAMATE TRANSPORTER VARIANTS GLT-1A AND GLT-1B IN THE CORTEX AND SPINAL CORD OF TRANSGENIC RATS EXPRESSING HSOD1G93A

GOURSAUD S, BERGER J, MALOTEAUX J-M, ERMANS E

Group of Neuropharmacology, Institute of Neurosciences, Université catholique de Louvain, Brussels, Belgium

E-mail address for correspondence: stephanie.goursaud@uclouvain.be

Keywords: glutamate transporter 1, splice variants, hSOD1G93A

Background: Loss of activity of the glutamate transporter GLT-1 and increased extracellular concentration of glutamate have been documented in sporadic and familial cases of amyotrophic lateral sclerosis (ALS) and transgenic rodent models expressing mutated human superoxide dismutase 1 (hSOD1). The cellular and molecular mechanisms causing the loss of GLT-1 expression have been largely investigated and identification of mutations in the GLT-1 gene and detection of aberrant GLT-1 transcripts in disease-affected areas have been reported. Interestingly, some experimental data indicate that the expression of the two C-terminus splice variants of GLT-1, namely GLT-1a and GLT-1b could be differentially and independently regulated in the motor cortex of ALS patients.

Objective: We herein characterized the GLT-1a and GLT-1b mRNAs and activity of the transporters at different symptomatic stages of the disease in the fronto-temporal cortex and in the lumbar spinal cord of a transgenic rat model expressing hSOD1^{G93A}.

Methods: Tissues were isolated from 60, 120 and 195 day old wild-type and transgenic animals. Number of copies of GLT-1a or GLT-1b mRNAs were obtained by quantitative real-time PCR. Activities of the transporters were evaluated in tissue synaptosomes by measuring D-[^{3}H]-aspartate uptake velocity in the presence of DHK, a selective GLT-1 blocker.

Results: GLT-1a transcripts were four-fold more abundant than GLT-1b transcripts in the fronto-temporal cortex of wild-type rats. During development of disease, expression of GLT-1a strongly decreased and expression of GLT-1b gradually increased so that abundance of the two transcripts was identical at the end stage of life (195 days) in the cortex of transgenic rats. Also, the cortical activity of GLT-1 decreased during progression of ALS. The quantity of GLT-1a and GLT-1b mRNAs was identical in the lumbar spinal cord of wild-type rats. In transgenic animals, GLT-1a and GLT-1b transcripts copy number were dramatically reduced in the ventral horn of the lumbar spinal cord of transgenic rats. In correlation with this loss of expression, activity of GLT-1 specifically decreased in this area.

Conclusions: Our results demonstrate that GLT-1a and GLT-1b are differentially expressed in the fronto-temporal cortex and the lumbar spinal cord of wild-type and transgenic animals. During the progression of the symptoms, expression of the mRNAs differentially and independently changed although GLT-1 activity constantly decreased in both structures.

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P24 REDUCING CASPASE-3 ACTIVITY UPREGULATES THE GLUTAMATE TRANSPORTER GLT-1A IN THE CORPUS CALLOSUM AND IN CULTURED CALLOSAL ASTROCYTES FROM A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (HSOD1G93A)

HERMANS E[1], FOCANT M[1], NIZET Y[2], BERGER J[1], MALOTEAUX J-M[1], GOURSAUD S[1]

[1]*Group of Neuropharmacology, Institute of Neurosciences* [2]*Institute of Experimental and Clinical Research; Université catholique de Louvain, Brussels, Belgium*

E-mail address for correspondence: emmanuel.hermans@uclouvain.be

Keywords: glutamate, transporter, caspase

Background: Upper motor neuron dysfunction in amyotrophic lateral sclerosis (ALS) has been associated with a deficit of transcallosal connections and a reduced volume of the corpus callosum. Besides, impairment of the astroglial glutamate transporter GLT-1 and related excitotoxicity are likely to participate in the progression of the disease. At the molecular level, caspase-3-mediated cleavage of GLT-1 leading to a selective inhibition of the transporter was evidenced in spinal cord homogenates of a transgenic mouse model of fALS. In addition, recent reports have documented alternative splicing mechanisms for GLT-1 and the differential expression of the isoforms GLT-1a and GLT-1b in ALS patients.

Objectives: We herein characterised the expression of GLT-1a and GLT-1b and the activity of the transporters after pharmacological inhibition of caspase-3 in callosal homogenates and in cultured callosal astrocytes isolated from transgenic rats expressing a mutated form of human superoxide dismutase 1 (hSOD1^{G93A}).

Methods: The experiments were performed in callosal homogenates isolated from symptomatic and wild-type rats and in cultured callosal astrocytes obtained from transgenic and wild-type newborn rats. The expression of GLT-1a and GLT-1b was characterized by quantitative RT-PCR and Western-blotting studies and the GLT-1 activity was determined in d-[^{3}H]-aspartate uptake assays in the presence of the inhibitor DHK. Caspase-3 activity was evaluated using the fluorogenic substrate Ac-DEVD-AMC. In some experiments, tissues were collected 48 h after intracallosal injection of 1 μg Peptide Histidine Isoleucine (PHI), a neuroprotective neuropeptide in the white matter.

Results: Differential expression of GLT-1a and GLT-1b was evidenced in the corpus callosum and in callosal astrocytes. Also, in the transgenic animals, GLT-1a was less abundant while robust expression of GLT-1b was observed. Local PHI instillation induced an upregulation of GLT-1a protein only in transgenic rats and specifically in the ipsilateral side. Expression of GLT-1b was unchanged. In these experimental conditions, activity of GLT-1 was also strongly upregulated. Similar results were obtained in cultured astrocytes treated with PHI or with Ac-Asp-Met-Gln-Asp-aldehyde, a selective inhibitor of caspase-3. Moreover, PHI also reduced caspase-3 activity after injection in the corpus callosum and in callosal astrocytes selectively in transgenic animals.

Conclusions: Reducing the activity of caspase-3 could exclusively upregulate GLT-1a in callosal astrocytes and in the corpus callosum of a transgenic rat model of ALS.

Supported by the FNRS, FMRE and ABMM.

P25 ABSENCE OF GFAP DOES NOT AFFECT DISEASE ONSET AND PROGRESSION IN SOD1H46R-EXPRESSING MICE

YOSHII Y[1,2], OTOMO A[2], LEI P[2], IKEDA K[1], IKEDA J-E[2], IWASAKI Y[1], HADANO S[2]

[1]*Department of Neurology, Toho University Medical Center Omori Hospital, Ota-ku, Tokyo, Japan,* [2]*Department of Molecular Life Sciences, Tokai University School of Medicine, Isehara, Kanagawa, Japan*

E-mail address for correspondence: yyoshii96097@aol.com

Keywords: GFAP, SOD1, astrocyte

Background: Interactions between glia and neurons have long been investigated in neuroscience fields. Glial fibrillary acidic protein (GFAP), vimentin and nestin are among the intermediate filaments (IFs) to form a group of cytoskeletal proteins. IFs play a role in the mechanical strength and shape of cells and their processes in physiological conditions. By contrast, in pathological conditions such as neurodegenerative disease, some of the IFs are up-regulated in activated astrocytes and are believed to have deteriorating factor for neuronal survival. Astrogliosis is one of the cardinal features for the progression of amyotrophic lateral sclerosis (ALS). Recent studies have demonstrated that glial cells carrying a human *SOD1*^{G93A} mutation adversely affect motor neuron survival with a non-cell autonomous trait in embryonic stem cell based ALS model, and expression of mutated SOD1 in rodent spinal astrocytes specifically contributes to neurodegeneration. Although GFAP is highly expressed in activated astrocytes and frequently used as a marker of them, it is still unclear whether GFAP by itself plays a role in the pathogenesis for ALS.

Objectives: To explore the effect of GFAP on disease onset and progression of ALS using a mutated SOD1-expressing mouse model.

Methods: We crossed two congenic lines; *Gfap*-knockout (KO) and *SOD1*^{H46R} transgenic mice on a C57BL/6 background, and generated mice with 6 different genotypes; *Gfap*+/+, *Gfap*+/-, *Gfap*-/-, *Gfap*+/+;*SOD1*^{H46R}, *Gfap*+/-;*SOD1*^{H46R}, and *Gfap*-/-;*SOD1*^{H46R}. We monitored body weight, disease onset, spontaneous motor activity (cage activity and rearing activity), and lifespan.

Results: No significant differences in the body weight, spontaneous motor activity, disease onset (160.3±1.47 days; mean±SEM), and survival (172.4±1.19 days; mean±SEM) were observed among *SOD1*^{H46R} mice with different *Gfap* genotypes.

Conclusions: GFAP by itself may play a limited role in the pathogenesis for the mutated SOD1-linked motor neuron disease. It is possible that other IFs, such as vimentin and nestin, are up-regulated for compensation, therefore analysis of these levels of these proteins is currently underway by Western blotting and immunohistochemistry.

P26 DO NEUROTOXIC INSULTS CAUSE MISFOLDING OF SOD1 IN AN ANIMAL MODEL OF SPORADIC ALS?

ZWIEGERS P[1], LEE G[1], LIU H-N[2], ROBERTSON J[2], SHAW CA[1]

[1]*University of British Columbia, Vancouver, British Columbia, Canada,* [2]*University of Toronto, Toronto, Ontario, Canada*

E-mail address for correspondence: pierre.zwiegers@gmail.com

Keywords: ALS-PDC, stigmasterol β*-D-glucoside, SOD1 conformation*

Background: Amyotrophic lateral sclerosis with missense mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) account for approximately 1-2% of all ALS cases, and is purported to be both clinically and neuropathologically indistinguishable from sporadic forms of the disease. The underlying pathobiological mechanism implicated in SOD1-mediated familial ALS (fALS) involves a toxic gain-of-function mutation with resultant misfolding and aggregation of the aberrant protein. The above observations, coupled with research demonstrating that both wild-type and mutant SOD1 can misfold and aggregate upon *in vitro* oxidation, has led to a hypothesis that aberrant SOD1 conformational changes underlie all ALS etiology. However, a recent study by Liu *et al* (2009) using the misfolded SOD1-specific SEDI antibody in sporadic and familial ALS cord samples does not support this interpretation.

Objectives: Previous work conducted by our group has shown that a Guamanian ALS-Parkinsonism dementia complex (ALS-PDC) phenotype can be replicated by exposing mice to stigmasterol β-D-glucoside (SG). Utilizing SOD1 conformation-specific antibodies, our studies attempted to determine if SG induced SOD1 conformational changes, as well as to probe for any synergistic effect on SOD1 misfolding arising from exposure of $mSOD^{G37R}$ mice to this toxin.

Methods: The human IMR-32 neuroblastoma cell line was exposed to SG at a concentration of 50 μM for a period of up to 9 days. Wild-type and transgenic $mSOD^{G37R}$ mice were treated with 42 μg/kilogram of body weight daily starting at 10 weeks of age. The role of SG regarding induction of aberrant SOD1 protein folding was investigated with the use of novel antibodies selective for epitopes indicative of misfolded SOD1.

Results: The results do not implicate SOD1 misfolding and aggregation as the mechanism of SG mediated neurotoxicity. Prolonged SG treatment of IMR-32 cells exhibited cell loss but failed to induce SOD1 misfolding. Additionally, SG exposure in mice did not demonstrate a synergistic interaction on SOD1 conformational state with the G37R mutation.

Discussion: Although the mechanism of SG toxin neuropathology is uncertain, it has been shown that dietary exposure to SG alone is sufficient to produce a disease phenotype and a more severe phenotype in conjunction with a genetic predisposition to ALS. These results posit that aberrant SOD1 folding is not implicated as a mechanism for SG neuropathology and support the notion that SOD1 conformational changes are unique to some forms of fALS.

Conclusion: In conclusion, the environmental agent studied here is not sufficient to induce or exacerbate SOD1 protein conformational changes. Through the use of novel conformation-specific antibodies we have demonstrated that the mechanism of SG neurotoxicity is similar to sALS in that misfolded SOD1 was undetectable. The environmental mouse model may be used to further understand the mechanism of disease progression in degenerative conditions exacerbated by environmental agents.

P27 OXIDATIVE MODIFICATION OF CYSTEINE 111 PROMOTES FORMATION OF DISULFIDE BOND-INDEPENDENT AGGREGATION OF SOD1

CHEN X[1], CUI L[2], QI X[2], SHANG H[3], KONG J[1]

[1]*University of Manitoba, Winnipeg, Canada,* [2]*Union Hospital, Beijing, China,* [3]*West-China Hospital, Chengdu, China*

E-mail address for correspondence: kongj@cc.umanitoba.ca

Keywords: oxidation, SOD1 aggregation, disulfide bond

Aggregation of mutant human SOD1 has been implicated in the 'gain of toxic property' that plays a role in the pathogenesis of ALS. To study the role of oxidative modification in SOD1 aggregation, we examined the redox state of SOD1 in the G37R transgenic mice at different stages of the disease.
Our data show that the cysteine residues in SOD1 from the spinal cord of G37R transgenic mice exhibited decreased accessibility to modification by malPEG with disease progression. This was at least partially caused by oxidative modification of the cysteine thiol groups because treatment of human SOD1 with H_2O_2 led to similar decreased accessibility to modification by malPEG as well.
Using an antibody (anti-C111ox-SOD1) specific for oxidized SOD1, C111-peroxidized SOD1 was found to be increased in abundance with the progression of the disease. Levels of oxidation correlated with the formation of SOD1 aggregation. Oxidation of both WT and Mutant forms of SOD1 with H_2O_2 induced formation of high molecular weight aggregates even in the presence of 5% β-mercaptoethanol, suggesting that SOD1 aggregation was not mediated by the disulfide bond between C111 and C6. Cysteine 111 existed in sulfhydryl state under normal circumstance compared with other cysteine residues. Endogenous mouse SOD1, which lacks C111, was not modifiable by malPEG. When cysteine 111 in human SOD1 was mutated to serine, it became unmodifiable by malPEG, while several other cysteine-residue-mutated SOD1s behaved just like wt SOD1 and were easily modifiable by malPEG.
The results suggest that cysteine 111 is a primary site of malPEG modification. Since malPEG can only react with the sulfhydryl group (-SH) of reduced cysteine residues, our data suggest that the sulfhydryl group (-SH) of cysteine 111 in human SOD1 is the target of thiolate anion (S-) for the oxidative modification, while other cysteine residues exist in disulfide (-S-S-) state.
In conclusion, cysteine 111 provides thiolate anion (S-) for oxidative modification. Oxidative modification of cysteine 111 promotes the formation of disulfide bond-independent aggregation of SOD1

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P28 THE CARBOXY-TERMINUS OF TDP-43 IS THE PRIMARY DETERMINANT OF ITS TOXICITY IN THE NERVOUS SYSTEM

HAZELETT D, STEWART J, MORTON D

Oregon Health & Science University, Portland, OR, United States

E-mail address for correspondence: hazelett@ohsu.edu

Keywords: TDP-43, Drosophila, cell biology

Background: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease resulting from the loss of upper and lower motor neurons in affected individuals. Recently TDP-43 was identified as the major constituent of cytoplasmic aggregates in motor neurons. TDP-43 is an highly conserved

RNA-binding protein normally expressed in the nucleus. In disease neurons, TDP-43 is excluded from the nucleus.

Objectives: We made overexpression transgenic lines and knockouts to test competing hypotheses that TDP-43 gain-of-function or loss-of-function might contribute to a motor-deficient phenotype.

Methods: We employed the yeast GAL4-UAS expression system to overexpress different isoforms of TDP-43 in larval and adult flies.

Results: TDP-43 knockouts do not eclose, but larvae have severely impaired locomotion. RNAi against TDP-43, which reduces expression about 2-fold, exhibits a significantly milder phenotype of age-dependent neurodegeneration. Overexpression of three naturally occurring c-terminal variant splice-forms of TDP-43 in the nervous system resulted in early lethality, depending on the c-terminus. Expression of the c-terminus alone had no effect. All three isoforms caused motor deficits when misexpressed with a motor-neuron driver, but the isoform lacking a c-terminus showed the mildest effect. All three isoforms also caused degeneration when expressed in photoreceptors, again with the c-terminal truncation having the mildest phenotype. In both cases overexpression of c-terminus alone had little or no effect. To compliment previously published analyses of neuromuscular junction (NMJ) anatomy in TDP-43 mutants, we examined NMJ in TDP-43 misexpressing larvae with motor neuron driver. Opposite to what was previously observed for loss of function, overexpression of all three isoforms promoted branching, outgrowth, and supernumerary bouton formation in Type IS sub-class of boutons but not Type IB.

Discussion: These data are not consistent with the model that TDP-43 overexpression causes a TDP-43 loss of function. However it remains possible that TDP-43 overexpression has pleiotropic effects that include its own loss-of-function in the nucleus. Together with the effects on motor neurons and photoreceptors, our findings indicate that the c-terminus is necessary, but not sufficient, for the toxic effects of TDP-43 in neuronal cells.

P29 DIFFERENTIAL EXPRESSION AND ALTERNATIVE SPLICING OF GENES IN THE LUMBAR SPINAL CORD OF SOD1-G93A TRANSGENIC MICE

GUO Y, CHEN H, HU M, ZHANG K, WANG Q, LI Z, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: exon array, gene expression, alternative splicing

Background: Amyotrophic lateral sclerosis (ALS) is one of the most common adult-onset neurodegenerative diseases, for which the exact pathogenic mechanism remains unknown. Recent evidence suggests that differential gene expression and alternative gene splicing may play a significant role.

Objectives: To identify differentially expressed genes and alternatively spliced exons in the lumbar spinal cord of SOD1-G93A transgenic mice at both presymptomatic and symptomatic stages.

Methods: Affymetrix GeneChip® Mouse Exon 1.0 ST Array was used to investigate the expression profiling and alternative splicing of genes in the lumbar spinal cord from both presymptomatic (30 days of age, 30A) and symptomatic (disease onset, about 120 days of age, 120A) SOD1-G93A mice and their non-transgenic littermates (30C and 120C, respectively). We selected transcripts that showed an increase/ decrease of at least two-fold or Splice Index (SI) ≥ 2 or ≤ 0.5 with a statistical significance ($P < 0.05$). Cluster analysis 3.0 software was used for hierarchical cluster (HC) analysis and gene ontology (GO) hierarchy analysis was then carried out. CapitalBio® MAS V4.0 software was used to identify known pathways. RT-PCR was performed to validate.

Results: The gene level analyses identified 263 (9.17%) up-regulated and 71 (2.47%) down-regulated genes in 120A vs 30A group and only one 2.8-fold up-regulated gene (with no annotation) in 30A vs 30C group. 322 (2.2%) genes were differentially expressed in 120A vs 120C group, of which 309 (95.96%) were up-regulated and 13 (4.04%) were down-regulated. The exon level analysis identified 63 alternatively spliced exons derived from 27 transcripts in 120A vs 120C group, 630 alternatively spliced exons derived from 563 transcripts in 120A vs 30A group, and 85 alternatiively spliced exons derived from 85 transcripts in 30A vs 30C group. Several differentially expressed genes (CD68, LPL, NOX_2, PIK3CG) and candidate splice variants (PDCD1, FYB) were validated with RT-PCR and consistency with exon array was found. Our findings indicate that cell adhesion, immune-inflammation response, lipid metabolism, and oxidative stress play important roles in the onset of mutant SOD1-related motor neuron degeneration.

Discussion: At 30 days of age, no significant change was found at both gene and exon levels between transgenic and non-transgenic mice, but 120-day transgenic mice differ significantly from their non-transgenic littermates and 30-day transgenic mice. The results suggest that a number of differentially expressed genes and alternatively spliced exons are involved in mutant SOD1-related disease onset of ALS. Functional and pathway analysis demonstrated the importance of transcription and splicing regulation in physiological and pathological processes.

Conclusions: We speculate that it is a multi-factor interactive mechanism that participates in the onset and progression of ALS, although much more remains to be understood.

P30 DIFFERENTIAL NEUREGULIN 1 ISOFORM EXPRESSION CORRELATES WITH MOTOR NEURON DEGENERATION AND GLIAL CELL ACTIVATION IN THE ALS-SOD1 MOUSE MODEL

SONG F, CHIANG P, LOEB J

Hiller ALS Clinic and Research Center, Department of Neurology, Center of Molecular Medicine and Genetics, Wayne State University, Detroit, MI 48201, United States

E-mail address for correspondence: fsong@med.wayne.edu

Keywords: neuregulin, neurodegeneration, glial activation

Background: Neuregulin 1 (NRG1) is a neuronally-expressed factor that supports axoglial and neuromuscular development. It can be regulated by neurotrophic factors derived from surrounding cells in both secreted (type I) and membrane-bound (type III) forms (1). To date, this important reciprocal signaling pathway has not been explored in mouse models of ALS. Since both NRG1 and neurotrophic factors are potent survival factors, understanding their normal function as well as their

altered function in models of ALS could lead to biologically-driven therapeutics to combat the progressive neuromuscular degeneration in ALS and other motor neuron diseases.

Objectives: To determine the relationship of NRG1 gene and protein expression with motor neuron loss and glial cell activation in spinal cords of SOD1 (G93A) mice compared to wild type (wt) littermate controls at serial life stages in order to develop novel therapeutic strategies in ALS.

Methods: Pathological changes were measured histologically for motor neuron number, astrocytosis, and microgliosis in both SOD1 mice and wt littermates at days 35, 56, 90 and end-stage (>day 117 but before death). Gene expression was determined by quantitative PCR for brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) and the NRG1 gene isoforms (Type I and Type III) that have important roles in nervous system development and compared to protein levels.

Results: Significant pathological changes including motor neuron loss**,** demyelination, astrocytosis and microgliosis were observed in spinal cords in SOD1 mice starting around or before 90 days. The development of these changes paralleled reciprocal changes in type I and type III NRG1 gene expression. While type I NRG1 was significantly upregulated, type III NRG1 was downregulated. GDNF and BDNF, known to induce both transcriptional as well as post-translational expression of NRG1 were mostly upregulated, even before periods of increased motor neuron loss. Studies defining NRG1 protein expression and distribution with respect to glial activation are currently underway. Through the regulation of NRG1 isofrom expression, neurotrophic factors may therefore play a regulatory role in glial activation and disease development in SOD1 mice.

Conclusions: Reciprocal changes in NRG1 isoform type expression and glial activation in the SOD1 model of ALS suggests a potential casual role for NRG1 in glial changes that occur together with motor neuron loss in this model. At the same time points, we observed changes in neurotrophic factor expression, known to regulate NRG1.

Discussion: The differential expression of NRG1 isoforms (membrane bound versus soluble) could be important either in the degeneration or repair process in ALS and therefore represent a potential therapeutic target by either up- or down-regulating NRG1 in ALS.

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P31 MICROARRAY ANALYSIS OF THE TRANSCRIPTOME FOLLOWING GLIA- AND MOTONEURON-SPECIFIC EXPRESSION OF MUTANT SOD1 IN DROSOPHILA

QUEEN E[1], GRISSOM J[2], CONWAY T[2], ZHANG B[1]

[1]*Department of Zoology, University of Oklahoma, Norman, OK, United States,* [2]*Department of Botany and Microbiology, Norman, OK, United States*

E-mail address for correspondence: Bing@ou.edu

Keywords: SOD1, Drosophila, microarray

Background: Mutations in the Cu/Zn superoxide dismutase (SOD1) gene account for about 20% of familial ALS cases. The normal function of SOD1 is to neutralize superoxide free radicals. Mutant SOD1 are thought to confer a toxic gain of function to the protein rather than loss of dismutase activity. However, the cellular and molecular mechanisms by which mutant SOD1 induces neurodegeneration remain unclear. Transgenic animals expressing mutant SOD1 are useful models for investigating ALS and have greatly enhanced our understanding the disease. Similar to the mouse model, transgenic *Drosophila* selectively expressing mutant SOD1 in motoneurons shows an age-dependent decline in motor behavior, defects in synaptic transmission, and the appearance of SOD1 protein aggregates.

Objectives: Although motoneurons are the affected tissue in ALS, research indicates that SOD1 linked ALS involves multiple cell types. Astrocytes and microglial cells, in particular, seem to contribute to the pathology attributed to mutant SOD1. The objective of this project is to investigate the contributions of glia and motoneurons in SOD1-linked defects in flies.

Methods: To fully understand the role of glia and motoneurons in ALS, we performed a large-scale screen to identify genes that are differentially expressed in mutant SOD1 flies. Using the UAS/GAL4 system, we generated flies expressing wild type *Drosophila* dSOD1 or mutant human SOD1 (G85R) in motoneurons, glia, and concurrently in motoneurons and glia. An Affymetrix GeneChip microarray was conducted on 5 and 45 day old flies expressing a mutant human SOD1 (G85R) and wild type *Drosophila* SOD1 in each of the above cell types.

Results: With the help of the bioinformatics team at the University of Oklahoma, we are analyzing genes that show a significant change in expression across the multiple groups. Preliminary analysis reveals detectable changes in many potentially interesting genes. For example, a 3 fold increase was observed in a neurotransmitter transport gene (CG33296) and a 4 fold decrease was detected in an oxidation reduction gene (CG18233), suggesting potential direct functional links with SOD1. Fully analyzed transcriptome data from these experiments will be presented in the meeting.

Discussion and conclusions: *Drosophila* is relevant to understanding human diseases because flies and humans share highly conserved genes and have similar cellular organization and function of the nervous system. Further, flies offer the advantage of a large and highly developed genetic toolkit that allows manipulations of specific genes in specific cell types. Several neurological diseases, such as Parkinson's and Alzheimer's, have been successfully modelled in flies and have provided important insights into human neurological disorders. A preliminary analysis of our microarray data has revealed similarities to microarrays conducted in mutant SOD1 mice and cell culture, illustrating the usefulness of a fly model of ALS.

P32 INVESTIGATION OF VESICLE TRAFFIC DEFECTS ASSOCIATED WITH MOTOR NEURON DEGENERATION IN WOBBLER MICE

SCHMITT-JOHN T, DROCE A, KARLSSON PR, ORTIZ-PADILLA C, MOSER JM

Molecular Biology Department, Aarhus University, Aarhus, Denmark

E-mail address for correspondence: tsj@mb.au.dk

Keywords: vesicle traffic, wobbler mouse model, motor neuron degeneration

Background: The wobbler mouse serve as an animal model for human ALS/MND since the wobbler phenotype has been

shown to closely resemble the human disease. We have identified the gene affected by the wobbler mutation to be the ubiquitously expressed vesicle traffic factor Vps54. Vps54 is a component of the GARP (Golgi associated retrograde protein) complex, a vesicle tethering factor involved in the retrograde vesicle traffic from endosomes to the Golgi apparatus and thus suggests a role for retrograde vesicle traffic in motor neuron degeneration.

Objectives: We investigated if vesicle traffic is affected in wobbler mice and analyzed the cellular effects of the Vps54 wobbler point mutation as well as Vps54 null mutations. Furthermore, we tried to find ways to investigate the retrograde vesicle traffic in human ALS/MND patients.

Methods: For this purpose, we utilized embryonic fibroblasts and embryonic stem cells from wobbler and Vps54 null mutant mice as well as cultured wobbler skin fibroblasts for the functional analysis of the retrograde vesicle traffic. Finally, we used cultured skin fibroblasts of human ALS patients in order to evaluate the option of screening ALS patients for wobbler-like defects in the retrograde vesicle traffic.

Results: In murine Vps54 mutant cells we observed an affected retrograde vesicle transport and mis-distributed mannose-6-phosphate receptors, while endocytosis was not affected. Similar experiments were performed with human skin fibroblasts but the variation from cell to cell was much higher in human skin fibroblast as compared to the murine cells.

Discussion and conclusions: Our results indicate that Vps54 and thereby the GARP complex is affected in wobbler mice and leads to a disturbance of retrograde vesicle traffic and thereby to mis-distribution of mannose-6-phosphate receptors in all cells tested so far. The vesicle traffic assays used for murine cells can be applied to human skin fibroblasts, even though the variability is much higher in human cells, probably due to the higher genetic variability as compared to mouse inbred strains. However, we think that a higher number of individual cells analyzed per patient will allow screening for vesicle transport defects among sporadic ALS patients.

P33 H63D VARIANT OF HFE ALTERS CHOLESTEROL METABOLISM: A POTENTIAL MECHANISM FOR DISRUPTION OF CELL SIGNALING IN AMYOTROPHIC LATERAL SCLEROSIS

ALI-RAHMANI F, LEE S, NANDAR W, NEELY B, SIMMONS Z, CONNOR J, SCHENGRUND C-L

Pennsylvania State University, Hershey, PA, United States

E-mail address for correspondence: fga101@psu.edu

Keywords: cholesterol, HFE, lipid rafts

Background: The HFE gene variant, H63D, has been under investigation as a risk factor for amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. Previous studies indicated that possession of at least one H63D allele is associated with a four-fold increased risk of ALS. HFE protein and glutamate transporters are localized in lipid rafts. Lipid rafts are microdomains in the plasma membrane and enriched in cholesterol and sphingolipids. Alterations in raft components can affect cell signaling functions of these proteins and can also affect cellular activities such as endo- and exocytosis. Reduction in membrane cholesterol has been shown to increase dissociation of the glutamate transporter from lipid rafts and causes loss of glutamate uptake. Recently, alterations in lipid metabolism have been observed in ALS patients and decreased glutamate uptake is reported in a cell model expressing H63D. The role of cholesterol metabolism in the pathogenesis of ALS is unclear but important to clarify as cholesterol-lowering agents are commonly prescribed.

Objectives: The aim of this study was to investigate the effect(s) of the H63D-HFE mutation on cholesterol metabolism and its association with ALS pathology.

Methods: We used SH-SY5Y human neuroblastoma cells transfected to stably express either wild type (WT) or the H63D variant of HFE. Total cholesterol content, targeted gene array analysis and protein expression was determined. In addition, brains were isolated from six and twelve month old mice expressing WT HFE and H67D (equivalent to H63D) and used for analysis of total cholesterol content and total protein expression.

Results: Analysis of cholesterol content indicated that cells expressing the H63D variant had 50% less cholesterol than cells expressing WT HFE. Targeted gene array analysis showed a six fold increase in the expression of cholesterol 24 hydroxylase (CYP46A1), an enzyme that converts cholesterol to 24S-hydroxycholesterol. Because sphingolipids are also key components of lipid rafts, we measured expression of mRNA for 13 genes involved in their metabolism. Transcription of genes encoding sphingosine kinase and GD3 synthase was reduced, suggesting alterations in sphingolipid metabolism. Analyses of 6 and 12 month old mice revealed that compared to WT-HFE expressing mice, homozygous H67D mice showed significant increase in the expression of cholesterol synthesis protein (DHCR24) and proteins regulating cholesterol efflux (CYP46A1, APOE, and ABCA1).

Discussion and conclusions: The H63D-HFE variant induces alterations in key lipid raft components including cholesterol and sphingolipids. This supports the hypothesis that disruption of lipid rafts induced by the HFE mutant may contribute to development of ALS. These data also have implications for the use of cholesterol-lowering agents to treat individuals with the H63D mutation.

P34 INCREASED HUMAN MUTANT SUPEROXIDE DISMUTASE 1 IN SPINAL CORD MITOCHONDRIA COMPARED WITH BRAIN MITOCHONDRIA AND SKELETAL MUSCLE MITOCHONDRIA IN THE RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

KUBALIK N, HEMENDINGER R, BROOKS BR

Carolinas Medical Center, Charlotte, NC, United States

E-mail address for correspondence: rhemendinger@carolinas.org

Keywords: superoxide dismutase, mitochondria, neurodegeneration

Background: Amyotrophic lateral sclerosis (ALS) is a destructive fulminate neurodegenerative disease with multifactorial pathogenesis including: mitochondrial dysfunctions, oxidative stress, SOD1 mutations, and protein misfolding. In animal models of ALS, the mutated human SOD1 (mtSOD1) protein may be associated with neuronal mitochondria (1). Spinal cord mitochondria from tgSOD1 rodents are more dysfunctional as compared with the brain mitochondria (2). Our laboratory has reported differences in metabolic activity and ROS generation between brain mitochondria and spinal cord mitochondria from wild-type and tgSOD1 (3,4). The reason for this dysfunction is unknown but may depend upon

the amount of mtSOD1 associated with between brain mitochondria or spinal cord mitochondria.

Objective: To examine the distribution of the human SOD1 expression in isolated mitochondria from different tissues from G93A-SOD1 transgenic rats in presymptomatic and symptomatic stages of ALS.

Methods: Brain, spinal cord, heart and skeletal muscle mitochondria were isolated from tgG93A SOD1 rats at three time points in the disease process: pre-symptomatic, at disease onset and at endstage. Mitochondria were isolated using the Mitosciences Mitochondria Isolation Kit for Tissue. Human SOD1 was quantified in rodent mitochondrial samples by immnoblotting and analyzed with General-Purpose Analysis Software Multi-Gauge V3.0. All rats were genotyped and copy number was controlled for these studies.

Results: Human SOD1 was detected in mitochondria from brain, spinal cord and muscle tissues, but within the central nervous system, the content of human SOD1 protein was 24.9% higher in spinal cord mitochondria in comparison with the brain mitochondria in pre-symptomatic stage. Human SOD1 was not increased in the mitochondria from skeletal muscle.

Conclusion: Our study supports the hypothesis that mitochondrial dysfunction may depend on the amount of mtSOD1 protein associated with mitochondria. The increased level of mtSOD1 in spinal cord mitochondria pre-symptomatically compared with brain mitochondria may result from SOD1 aggregation in spinal cord mitochondria leading to neurodegeneration in motor neurons.

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P35 THE ROLE OF ACTIVITY IN NEUROMUSCULAR SYNAPTIC DEGENERATION: INSIGHTS FROM *WLD^S* MICE

BROWN R, THOMSON D, SHEWARD J, HARTLEY R, HARMAR A, RIBCHESTER R

University of Edinburgh, Edinburgh, United Kingdom

E-mail address for correspondence: s0459199@sms.ed.ac.uk

Keywords: neuromuscular junction, degeneration, activity

Background: Evidence for the role of activity in the onset or progression of Motor Neuron Disease (MND/ALS) is controversial. Some patients perceive benefits from taking exercise but conflicting evidence has raised the alternative possibility that some forms of activity may constitute a risk factor for onset of ALS or accelerate disease progression, perhaps through its potentially excitotoxic effects. The neuromuscular junction (NMJ) is the first component of the motor neuron to degenerate in some forms of MND, including the SOD1G93A mouse model and sporadic ALS in humans.

Objectives: Since exercise directly regulates neuromuscular activity, we asked whether blocking or enhancing activity either suppresses or accelerates synaptic degeneration at neuromuscular junctions induced by interruption of axonal transport. To test this, we used mutant *Wld^S* mice with slow-Wallerian degeneration in which axons are preserved but synapses degenerate progressively over several days after nerve section (1).

Methods: To study effects of paralysis, chronic sciatic nerve block was initiated in *Wld^S* mice and sustained for one week using microcapsules filled with tetrodotoxin (TTX; 10 mM). Following tibial nerve axotomy, neuromuscular synaptic preservation was measured physiologically 3-5 days later by intracellular recording from muscle fibres in isolated flexor digitorum brevis nerve-muscle preparations. The prevalence of fibres showing spontaneous (MEPPs) and/or evoked responses (EPPs) were scored in each muscle. To facilitate activity instead, *Wld^S* mice were individually housed and given voluntary access to running wheels for 2-4 weeks, before cutting the sciatic nerve. *Wld^S* mice showed a normal circadian rhythm under open-field conditions and typically ran about 13 km per night when provided with running wheels. Functional innervation was then assayed 3-5 days after axotomy in these mice as well. In some mice (transgenic thy1.2YFP16/Wld^S), isolated lumbrical muscles were stained with rhodamine-conjugated a-bungarotoxin and fluorescence microscopy was used to score the numbers of innervated and denervated motor endplates.

Results: The results showed that priming motor nerve terminals in *Wld^S* mice by chronic TTX-induced nerve conduction block significantly accelerated synaptic degeneration compared to controls (68% unresponsive fibres in the TTX pretreated group compared to 21% in axotomised control groups; $P<0.05$; ANOVA). Wheel-running liminally increased muscle fibre diameter and EPP amplitude in *Wld^S* mice. However, the number of denervated fibres observed 3-5 days after axotomy was neither increased nor decreased following up to a month of voluntary wheel running.

Discussion and conclusions: The data suggest that neuromuscular disuse suppresses the neuroprotective effect of the *Wld^S* gene and enhances neuromuscular synaptic degeneration following challenges that disrupt axonal integrity. However, environmental enrichment by facilitated aerobic exercise neither enhanced nor suppressed the protective effect of *Wld^S* on synaptic degeneration. We are presently extending this evaluation to SOD1G93A transgenic mice.

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P36 MUSCLE SATELLITE CELL BEHAVIOUR IN A MOUSE MODEL OF ALS

MANZANO R[1], TOIVONEN J[1], FERNANDES F[1], CALVO A[1], OLIVÁN S[1], MONTARRÁS D[2], UCKINGHAM M[2], MUÑOZ JM[1], ZARAGOZA P[1], RODELLAR C[1], OSTA R[1]

[1]*LAGENBIO-I3A, Facultad de Veterinaria, Instituto Aragonés de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain,* [2]*Unité de Génétique Moléculaire du Développement, Institut Pasteur, Paris, France*

E-mail address for correspondence: accalvo@unizar.es

Keywords: muscle, satellite cells, culture

Background: Skeletal muscle satellite cells are the main cells implicated in regenerative response following muscle injury. Although ALS is described as a disease related to upper and lower motor neuron degeneration, the first events described in ALS are related to neuromuscular junction perturbation

suggesting that an impairment of the muscle could produce toxic signals that would destroy neuromuscular junctions leading to a progressive retrograde axonophathy or 'dying-back'. If this is the case it is of interest to study the skeletal muscle satellite cell status and regenerating response throughout the disease.

Objectives: The aim of this study was to analyze behavior of satellite cells in the ALS mouse model hSOD1-G93A in the course of the disease.

Methods: Skeletal muscle satellite cells were isolated from fast and slow type muscles of hSOD1G93A and wildtype male and female mice of ages corresponding to 7, 40, 60, 90 and 120 days. Cell cultures were established and proliferation was measured by fixing the cultures every 24 hours, staining the nuclei of cells with Hoechst 33342 and recording the fluorescence intensity with a plate reader. Myogenic regulatory factors Pax7, Myod1 and Myogenin expression was obtained by Real Time PCR at the same timepoints.

Results: Proliferation ratio differences were found between transgenic and wildtype mice as well as between both sexes and fiber type muscles. Myogenic regulatory factor RNA levels showed that the expression of those factors were also affected; presenting sex, age and fiber-type dependent differences.

Conclusions: Muscle satellite cell proliferation and myogenin regulatory factor expression pattern are affected in the hSOD1G93A ALS mouse model. Further experiments are needed to confirm and determine the cause and pathogenic effects of these findings.

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P37 CGRP IMMUNOREACTIVITY LEVELS DIFFERENTIATE ALS-VULNERABLE FROM ALS-RESISTANT MOTONEURONS IN SOD1-G93A MICE

RINGER C, WEIHE E, SCHÜTZ B

Institute of Anatomy and Cell Biology, Faculty of Medicine, Philipps-University, Marburg, Germany

E-mail address for correspondence: schuetzb@staff.uni-marburg.de

Keywords: CGRP, vulnerability, immunohistochemistry

Background: In ALS, motor nuclei in brain stem and spinal cord are affected unequally severe by the disease. While some nuclei (eg Ncll. III, IV and VI) are resistant and largely spared from neuropathology, others (e.g. Ncll. V, VII, XII and spinal cord) are vulnerable but show different levels of motoneuron loss. The reasons for this selective resistance and vulnerability are still poorly understood. Calcitonin gene-related peptide (CGRP) is a neuropeptide co-expressed by some but not all motoneurons (1). Recently, we described disease-related changes in the sub-cellular distribution pattern of the ß-isoform of CGRP in the lumbar spinal cord of SOD1-G93A mice (2). The atypical appearance of ßCGRP in motoneuron dendrites and their close association with reactive astrocytes suggested a role for ßCGRP in the pathology of ALS.

Objective: In the present report we questioned if there is a connection between CGRP expression levels and the vulnerability of motoneurons during ALS pathology.

Methods: Lumbar spinal cord and brain from SOD1-G93A and wild type littermates at age P140-160 (n=3 each) were analyzed by double-immunoflurescence for CGRP and the motoneuron marker choline acteyltransferase (ChAT). For each area of interest all ChAT-positive neurons with clearly cut nucleus were counted on 10 sections and then divided in three groups: non CGRP (ChAT-staining only), low CGRP (weak, diffuse CGRP-staining) and high CGRP (strong, reticular network staining) expressing motoneurons.

Results: In wild type mice, the motor nuclei could be grouped into three clusters that differed in their amount of CGRP co-expression: The first cluster consisted of Ncll. III, IV and VI and contained only very few (<1%) high CGRP, 10-20% low CGRP and 80-90% non CGRP motoneurons. The second cluster (Ncll. V, VII and XII) contained about 30% high CGRP, 40% low CGRP and 30% non CGRP expressing neurons. Finally, Ncl. ambiguus and the lumbar spinal cord contained about 80% high CGRP expressing neurons, 10% low CGRP and 10% non CGRP expressing motoneurons each. While in the first cluster no (VI) or only 20% (III, IV) of all motoneurons had died at end stage in SOD1-G93A mice, the second type lost nearly 50% and the third type about 70% of motoneurons. Motoneuron loss was largely attributable to the level of CGRP expression. On average, high CGRP expressing motoneurons were found reduced by 80%, low CGRP expressing motoneurons by 50%, whereas the number of non CGRP motoneurons was not significantly affected.

Conclusions: Our analysis revealed CGRP immunoreactivity levels as a marker and/or criterion for the selective vulnerability of subsets of motoneurons within distinct motor nuclei during neuropathology in the SOD1-G93A mouse model of ALS.

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P38 UNDERSTANDING THE ROLE OF MACROPHAGE AND SCHWANN CELLS IN PERIPHERAL NERVES OF THE SOD1 MOUSE MODEL OF ALS

WANG M, MOY P, SANCHEZ R, CARRION I, PERRIN S, LINCECUM J

ALS TDI, Cambridge, MA, United States

E-mail address for correspondence: jlincecum@als.net

Keywords: macrophage, Schwann cells, SOD1

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease of the upper and lower motor neurons. The disease mechanism has yet to be fully defined. A hallmark of ALS pathology, in the peripheral nerves, is neuromuscular junction orphaning and demyelination suggesting a chronic injury state. It is well understood that, in response to trauma, peripheral nerves secrete cytokines and chemokines. This potent class of proteins has immunoregulatory and chemoattractive functions that promote a balanced inflammatory and anti-inflammatory response. We have previously established that macrophages accumulate in the peripheral nerves of gastrocnemius tissue as a function of disease progression. This was demonstrated by increased CD68(+) macrophage accumulation in the distal nerves of SOD-G93A transgenic mice relative to age-matched wild-type peripheral nerves.

Here we report on our efforts to further characterize the individual cell population in the peripheral nerves of SOD1-G93A mice. Focusing on macrophage and Schwann cells, we surveyed a panel of antibodies. This survey showed the cell surface protein, galectin-3/Mac2, was expressed on macrophage

and Schwann cells in a disease specific pattern. Galectin-3 is a beta-galactosidase binding protein that has been shown to be involved in the inflammatory pathway, specifically in the transition from acute to chronic inflammation. Using immunoblot analysis and quantitative ELISA we show that galectin-3 protein levels are increased throughout disease progression whereas no significant levels are seen in non-transgenic control mice. Furthermore, high resolution, 3-D imaging in muscle tissue revealed extensive galectin-3 expression in distal nerves, specific to CD68+ macrophage and a subset of Schwann cells. To characterize these two cell populations we developed a paramagnetic bead based purification strategy to isolate galectin-3 postive macrophage and Schwann cells. Subsequently, these purified cells were analyzed by transcriptional profiling which established the activation of several inflammatory pathways, such as the TLR pathway, associated with chronic injury. These studies support a role for demyelination and presympotomic peripheral nerve damage in ALS.

P39 NEUROPATHOLOGY OF MICE EXPRESSING A VERY LOW COPY NUMBER OF THE MUTANT HUMAN G93A SOD1 GENE ASSOCIATED WITH ALS

DEITCH J[1], MASEL R[2], KOCHER K[3], HEIMAN-PATTERSON T[1]

[1]*Drexel University College of Medicine, Philadelphia, PA, United States,* [2]*Muhlenberg College, Allentown, PA, United States,* [3]*Dickenson College, Carlisle, PA, United States*

E-mail address for correspondence: jdeitch@drexelmed.edu

Keywords: SOD1, copy number, pathology

Background: Transgenic mice expressing multiple copies of the human mutant SOD1 gene develop motor neuron (MN) pathology and clinical symptoms that are similar to patients with ALS/MND. In mice with 24 copies of the transgene, MN disease is evident as early as 30 d old and degeneration proceeds rapidly. This time course makes it difficult to discern cellular events at onset of pathology. We have developed a line of transgenic mice, expressing a very low number (4-5) of copies of the G93ASOD1 transgene that do not show clinical signs until 650 d old, if at all.

Objective: To identify MN pathology occurring sub-clinically in very low G93ASOD1 expressing (VLE) mice and determine whether onset of pathology begins early and progresses very slowly or if MNs are healthy until onset later in life.

Methods: Four male and four female mice each at approximately 250, 500 and 750 d old were selected. At each age and gender, two were VLE mice and two were non-transgenic littermates. The number of G93ASOD1 gene copies was confirmed by qPCR. MNs in the ventral horns of lumbosacral segments were counted. MNs were easily identifiable by their position and large (>30 μm) cell bodies. The total numbers of MNs in transgenic vs control mice were compared using a paired Student's t-test.

Results: There was no statistically significant difference in mean number of MNs in lumbosacral spinal cord at 250 days old ($P > 0.2$, one-tailed t-test with similar variances) between transgenic (2390±411, mean±std. dev.) and non-transgenic (2149±641) mice. Similar MN counts were found in non-transgenic (2212±492) and transgenic (1908±28) mice at 500 days old ($P>0.2$). At 750 days old, the number of lumbosacral MNs in non-transgenic mice (2296±233) is comparable to that seen at younger ages, however, the number is significantly reduced by 38% in transgenic mice (1431±63; $P<0.02$). This reduction is comparable to that seen at 70 d old in the transgenic mice expressing a high copy number of G93ASOD1 genes. The loss of MNs was most noticeable in the lateral motor column.

Discussion and conclusions: The mechanisms by which mutant SOD1 is toxic to MNs is still unclear. In the transgenic mouse model, 24 copies of the mutant gene result in rapid MN loss, making fine distinction between early and late phenomena difficult. In this study we demonstrated that MNs in VLE mice do not degenerate in significant numbers until late in life, but that neurotoxic events occurring much earlier may be studied distinct from cell loss. These data also support the concept that accumulation of mutant SOD1 protein results in cell death when it reaches a critical level, which occurs more slowly with fewer copies of the gene.

P40 AUTONOMIC IMPAIRMENT IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

KANDINOV B[1], KORCZYN A[1], RABINOWITZ R[1], NEFUSSY B[2], DRORY V[2]

[1]*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel,* [2]*Department of Neurology, Tel-Aviv Medical Center, Tel-Aviv, Israel*

E-mail address for correspondence: vdrory@post.tau.ac.il

Keywords: autonomic impairment, SOD1 transgenic mice, sympathetic activity

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons, but it is increasingly recognized that non-motor manifestations may occur. The autonomic nervous system may also be affected. To better understand the autonomic involvement in ALS we measured autonomic functions in transgenic (TG) mice carrying a SOD1 (G93A) mutation and wild-type (WT) control mice. TG mice had a higher heart rate at rest and following stress than WT mice at all ages except for the advanced stages of the disease (19-20 weeks of age). The mean pupil diameter at rest was similar in WT and TG mice; however, TG mice had decreased mydriasis following administration of morphine. The rectal temperature did not differ between TG and WT mice at rest, during exposure to cold stress and following administration of morphine (30 mg/kg) except for the advanced stages of the disease in which TG mice had significantly lower temperatures than WT mice during cold stress and following morphine administration. The results suggest mainly sympathetic cardiac hyperactivity, but also parasympathetic nervous system impairment in this ALS model, consistent with clinical data in humans.

P41 CONTINUOUS NON-INVASIVE INFRARED MOTION SENSING AS A MEASURE OF NEUROLOGICAL DISEASE PROGRESSION IN THE SOD1-G93A TRANSGENIC MOUSE MODEL OF ALS

DHILLON H, REED W, THOMPSON K, KIDD J, AL-NAKHALA B, VIEIRA F, GILL A

ALS TDI, Cambridge, MA, United States

E-mail address for correspondence: jlincecum@als.net

Keywords: SOD1-G93A, activity, automation

Background: The SOD1-G93A transgenic mouse manifests all clinical symptoms of sporadic and familial ALS. These

animals display an ALS-like phenotype and pathology, including muscle atrophy and motor neuron degeneration. Understanding disease progression in this model is a requirement for drug discovery leading to ALS therapies. ALS-TDI has developed a four-point neurological scoring system (1-4, 0= no neurological signs) that tracks the progression of ALS in these mice. While neurologic scoring (NS) is robust and reproducible, it involves a single observation point per day. Less discreet, gradual changes in neurological disease progression are unlikely to be captured using this method. There is a clear need for a more sensitive method for monitoring disease progression in SOD1-G93A mice in order to allow better assessment of new therapeutics.

Objectives: The aim of the current study was to design, build, develop and test a home-cage continuous monitoring system, and relate mouse activity over time to neurologic disease progression in this ALS model. The longer-term objective is to refine the system for a large-scale use to enhance sensitivity of neurological disease progression tracking during survival.

Methods: A novel Animal Movement Sensing System (AMSS) was built to continuously capture activity levels in each mouse's home cage. Continuous activity data (counts/min) were captured for 32 animals from 50 days to death. Experimental groups were age-matched SOD1-G93A mice (male and female, n=11 each) and WT mice (male and female, n= 5 each). Mice were individually housed in cages fitted with Passive Infra Red (PIR) sensors. The cage sensor nodes consist of two PIR sensors connected to Delta Sigma data converters, which connect to a microcontroller. The firmware read the output of the data converters and processed it to detect motion events from the raw sensor data. Motion events were counted by the microcontroller and stored in memory until requested by the computer.

Results and conclusions: Our data showed a significant difference in activity patterns from SOD1-G93A vs WT mice. This activity difference was consistent across genders and correlates well with neurologic disease progression as assessed by our manual scoring system. We observed a decline in activity counts in day 100-120 SOD1-G93A mice vs day 80-100 SOD1-G93A mice (59% males, 35% females). In addition to detecting gradual loss in overall mobility during disease progression, our AMSS accurately captured the diurnal periodicity of mouse activity. These data are the first documentation of continuous activity monitoring of SOD1-G93A transgenic mouse in their home cage. In conclusion, we have generated a simple and robust motion monitoring system that can be scaled up to thousands of sensors for large scale testing. This system will prove highly valuable in testing new drugs in preclinical mouse models of ALS.

P41A DISRUPTED TGF-BETA SIGNALING IN SPINAL AND BULBAR MUSCULAR ATROPHY

KATSUNO M[1,2], ADACHI H[1], MINAMIYAMA M[1], DOI H[1], KONDO N[1], BANNO H[1], SUZUKI K[1], TANAKA F[1], SOBUE G[1]

[1]*Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan,* [2]*Institute for Advanced Research, Nagoya University, Nagoya, Japan*

E-mail address for correspondence: ka2no@med.nagoya-u.ac.jp

Keywords: spinal and bulbar muscular atrophy, TGF-beta, androgen receptor

Background: Spinal and bulbar muscular atrophy (SBMA) is a late-onset lower motor neuron disease caused by the expansion of a trinucleotide CAG repeat, which encodes a polyglutamine tract in the androgen receptor (AR). Although it is commonly held that the pathogenic polyglutamine proteins accumulate in neurons and thereby induce transcriptional dysregulation through inhibition of histone acetylation, the down-stream molecular events have remained elusive.

Objectives: The aim of this study is to elucidate the molecular events that induce neurodegeneration in SBMA. Since a cDNA microarray study of cultured cells suggests that the expression of genes involved in transforming growth factor-beta (TGF-beta?) pathway is specifically regulated by histone acetylation, we examined whether TGF-beta signaling is dysregulated in SBMA.

Methods: We used a transgenic mouse carrying human AR with 97 CAGs and SH-SY5Y cells expressing truncated AR fragment with 97 CAGs, as mouse and cellular models of SBMA. Histopathological analysis was performed on the autopsy specimens of the spinal cord from SBMA patients and that from the model mice. TGF-beta signal transduction in cells and mice was analyzed using immunoblotting, immunoprecipitation, filter trap assay, RT-PCR and immunohistochemistry. Cellular toxicity analysis was performed using propidium iodide staining and WST-1 viability assay. Promoter activity was measured using luciferase reporter assay.

Results: Nuclear translocation of phosphorylated Smad2/3, a key step in TGF-beta signaling, is suppressed in the spinal motor neurons of male transgenic mice carrying the mutant human *AR*. A similar finding was also observed in the motor neurons, but not in Purkinje cells, of SBMA patients. The pathogenic AR, the causative protein of SBMA, inhibits the transcription of TGF-beta receptor type II (TbetaRII), via abnormal interactions with NF-Y and p300/CBP-associated factor. Furthermore, overexpression of TbetaRII dampens polyglutamine-induced cytotoxicity in a neuroblastoma cell line expressing the pathogenic AR.

Discussion: It was postulated that TGF-beta signaling plays a fundamental role in neural activity through the regulation of synaptic function. TGF-beta was also shown to protect neurons from glutamate-mediated excitotoxicity, a putative molecular mechanism underlying the pathogenesis of motor neuron diseases. Our findings indicate that the decreased expression of TbetaRII and the resulting perturbation of TGF-beta signaling appear to underlie polyglutamine-dependent neurodegeneration in SBMA. Decrease in TbetaRII expression was also reported in AD patients, suggesting that this molecule plays an important role in various pathogeneses of neurodegeneration.

Conclusion: The present study showed that polyglutamine-dependent neuron damage in SBMA is associated with the disruption of TGF-beta signaling due to transcriptional dysregulation of TbetaRII. Our findings further suggest that restoration of the brain TGF-beta-Smad2/3 pathway might be a potential therapeutic approach to polyglutamine-induced neurodegenerative diseases.

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THEME 3 *IN VITRO* EXPERIMENTAL MODELS

P42 SIGNALING PATHWAYS INVOLVED IN SBMA PATHOGENESIS

SARMA T, BRADY S

University of Illinois at Chicago, Department of Anatomy and Cell Biology, Chicago, IL, United States

E-mail address for correspondence: tsarma@uic.edu

Keywords: SBMA, flutamide, fast axonal transport

Background: Spinal and bulbar muscular atrophy (SBMA) is an inherited neurodegenerative disorder caused by expansion of a CAG repeat (glutamine) in the gene encoding the androgen receptor (AR). The role of polyglutamine expansion in human AR function and SBMA pathogenesis remains unclear. AR is a transcription factor that translocates to the nucleus in the presence of the AR ligand testosterone. The polyglutamine-expanded AR (polyQ-AR) is proposed to alter transcription after ligand binding and contribute to pathogenesis. However, polyQ-AR also alters cytoplasmic signaling pathways and inhibits fast axonal transport (FAT), leading to reduction or mistargeting of critical components delivered by FAT.

Objectives: In this study, we analyzed the effects of androgen agonists and antagonists in a SBMA cell model.

Methods: We evaluated the relative contribution of androgen binding and polyQ-AR translocation to the nucleus to effects on cell morphology, activation of c-Jun N-terminal kinases and FAT.

Results: Using androgen antagonists we compared the role of ligand-dependent nuclear versus cytoplasmic signaling. Interestingly, we found that the androgen antagonist, flutamide, restores FAT in squid axoplasm and modifies the cellular phenotype of SBMA pathogenesis, while inhibiting translocation of AR to the nucleus. In contrast, a second androgen antagonist, cyproterone acetate alters nuclear translocation, but does not prevent polyQ-AR effects on FAT or cell morphology. Moreover, androgen antagonist flutamide, changed the subcellular localization of phospho-JNK in cells expressing polyQ-AR. This indicates that androgen signaling is not critical for JNK activity.

Discussion and conclusions: These studies suggest that polyQ-AR-induced changes contribute to neuropathology through a cytoplasmic signaling pathway and that nuclear signaling is not required for polyQ-AR-induced pathology.

P43 IDENTIFICATION OF FUS INTERACTING PROTEINS IN NEURONAL CELLS

FUMOTO K[1], GROEN E[2], VAN DEN BERG LH[2], PASTERKAMP J[1]

[1]*Department of Neuroscience and Pharmacology,* [2]*Department of Neurology; Rudolf Magnus Institute of Neuroscience, Utrecht, Netherlands*

E-mail address for correspondence: k.fumoto@umcutrecht.nl

Keywords: FUS, NSC34 cells, protein-protein interaction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder, selectively affecting motor neurons and pathologically characterized by the presence of intracellular ubiquitin (Ub)-positive aggregates. TDP-43 has been identified as one of the major constituents of Ub aggregates in ALS and its dysfunction is thought to contribute to the neurotoxicity underlying the ALS neurodegenerative process. More recently, missense mutations in FUS (fused in sarcoma, also known as TLS) were identified and associated with the pathogenesis of familial ALS. FUS is an RNA binding protein and regulates RNA processing, DNA repair, nucleocytoplasmic shuttling, and axonal trafficking in neurons. Like TDP-43, mutations in FUS cause protein mislocalization and induce cytoplasmic aggregation. We recently analyzed mutations in the coding region of the FUS gene in a cohort of Dutch familial ALS patients. Two previously identified mutations (R521C and R521H) and one novel mutation (S462F) were found. Additionally, a Q210H polymorphism was identified in one proband and three healthy controls. However, the functional consequences of these novel mutations await analysis.

To analyze the cellular localization of FUS, we tagged FUS (wild type, R521C, S462, or Q210H) with enhanced green fluorescence protein (GFP) at the N-terminus and expressed the fusion proteins in COS7 and NSC34 cells. Wild type GFP-FUS was confined to the nucleus, resembling the endogenous FUS expression pattern. In contrast, the R521C mutant was mainly distributed in the cytoplasm and protein aggregations were frequently observed. Q210H and S462F were mostly confined to the nucleus although a weak cytoplasmic distribution was observed. To further understand the cellular function of FUS in motor neurons, biotinylation (Bio) peptide-tagged GFP, wild type GFP-FUS or GFP-FUS mutants (S462F, R521C) were transiently co-expressed with BirA enzyme in NSC34 cells so that Bio-tagged proteins were biotinylated in cells. Then, the lysates were pulled down with streptavidin beads and samples were size-separated. Gels were stained with silver to visualize differential bands followed by whole lane sequencing by Mass-spectrometry. The effect of mislocalization of mutated FUS on cell viability and toxicity through the newly discovered interacting proteins will be discussed.

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P44 DEVELOPMENT OF A NOVEL NON-RADIOMETRIC ASSAY FOR NUCLEIC ACID BINDING TO TDP-43 SUITABLE FOR HIGH-THROUGHPUT SCREENING USING ALPHASCREEN TECHNOLOGY

PAWLYK A, CASSEL J, BLASS B, REITZ A

ALS Biopharma, LLC, Doylestown, PA, United States

E-mail address for correspondence: apawlyk@alsbiopharma.com

Keywords: TDP-43, high-throughput screen, TAR DNA

Background: TAR DNA binding protein 43 (TDP-43) is a nucleic acid binding protein that is associated with the pathology of amyotrophic lateral sclerosis (ALS), the related disorder frontotemporal lobar dementia, certain forms of cystic fibrosis, and HIV infection. Assays to examine nucleic acid binding to TDP-43 typically use qualitative techniques that are not amenable to the high-throughput screening techniques necessary to discover small molecule probes or therapeutics.

Methods: We have developed a robust, quantitative, non-radiometric high-throughput assay measuring oligonucleotide binding to TDP-43 using AlphaScreen technology. AlphaScreen technology works by detecting the proximity of TDP-43 tethered to an acceptor bead and a DNA oligonucleotide tethered to a donor bead. This permits energy transfer via excited singlet oxygen when the two macromolecules are associated. We have established this assay in a 384-well plate format in which beads and macromolecules are pre-incubated in separate reactions and then combined in the presence or absence of a competing molecule. This allows the assay to detect direct binding of biotinylated oligonucleotides or competitive binding of a test oligonucleotide or small molecule. A chemical diversity library of 7,360 predominantly heterocyclic drug-like molecules was screened for their ability to disrupt this interaction.

Results: Biotinylated single-stranded TAR DNA (bt-TAR-32) and six TG repeats (bt-TG6) bound with high affinity to TDP-43, with K_D values of 0.75 nM and 0.63 nM, respectively. Both oligonucleotides exhibited slow dissociation rates, with half-lives of 750 min for bt-TAR-32 and 150 min for bt-TG6. The relative affinities of unlabeled DNA and RNA oligonucleotides, as determined by displacement of either bt-TAR-32 or bt-TG6, were consistent with previous reports of nucleic acid interactions with TDP-43, where increasing TG or UG repeats yields greater affinity. We also found that DNA oligonucleotides bound with a greater affinity than RNA oligonucleotides. Screening the library of 7,360 compounds for inhibition of TDP-43 binding to bt-TAR-32 identified a series of compounds with nascent SAR and IC_{50} values ranging from 100 nM to 10 μM.

Discussion and conclusions: We have established a homogenous, quantitative, high-throughput assay for the binding of nucleic acids to TDP-43 and demonstrated that this assay can be used to assess both direct and competitive binding interactions. We have demonstrated it is capable of identifying small molecule inhibitors of the nucleic acid-TDP-43 interaction from compound libraries. These compounds may prove to be useful biochemical tools to facilitate the elucidation of the function of TDP-43 and may lead to novel therapeutics for indications where the TDP-43-nucleic acid interaction is causal to the associated pathology. Screening of additional compound libraries and the development of downstream assays of TDP-43 function will enhance our understanding of the pathogenic role of TDP-43 in ALS and other diseases.

P45 INHIBITION OF SIRT1 FUNCTIONS PROMOTES NEURAL PROGENITORS TOWARD MOTONEURON DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS

ZHANG Y[1], WANG J[1], CHEN G[2], FAN D[1], DENG M[1]

[1]*Department of Neurology,* [2]*Reproductive Center; Peking University Third Hospital, Beijing, China*

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: Sirt1, resveratrol, nicotinamide

Background and objectives: A few protocols of directing embryonic stem cells (ESCs) to differentiate into functional motoneurons have been established, but the efficiency of motoneuron generation varied from different human ESC lines. We tried to search a novel protocol to increase the formation of motoneurons from ESCs.

Methods: In this study, we tested a nuclear protein, histone deacetylase Sirt1 to influence neural precursor cells (NPCs) development during differentiation of hESCs into motoneurons. A specific inhibitor of Sirt1, nicotinamide dramatically increased motoneuron formation.

Results: We found that about 60% cells from the total NPCs express HB9 and βIII-tubulin proteins, the typical motoneuron markers derived from ESCs after nicotinamide treatment. A functional marker of mature motoneurons, choline acetyltransferase (ChAT) was found positive from the derived cells. The inhibition of Sirt1 promotes more motoneurons (59.7%) to differentiate from a human embryonic stem cell line PKU1.1 than that of traditional protocol (32.8%) which used a simple sequential application of retinoid acid (RA) and sonic hedgehog (SHH) in a chemically defined suspension culture. Moreover, we also examined the transcript levels of Mash1, Ngn2, and HB9 in the differentiated NPCs treated with the Sirt1 activator resveratrol (50 μM) or inhibitor nicotinamide (100 μM) for 6 days by a quantitative RT-PCR, respectively. The levels of Mash1, Ngn2, and HB9, mRNAs are consistently increased significantly after nicotinamide treatment compared with control groups which used the traditional protocol. On the contrary, the levels of Mash1, Ngn2, and HB9 mRNAs by resveratrol treatment were all significantly lower than that of control group.

Conclusions: Our results suggested that increase of Mash1 and Ngn2 levels by inhibition of Sirt1 could elevate HB9 expression, which promote motoneuron differention. This study provides an alternative method for the production of sufficient amounts of engraftable motoneurons, a key requirement in the development of hESC-based cell therapy in motoneuron disease.

P46 GRADIENT CENTRIFUGATION ENRICHMENT OF MOTOR NEURONS DERIVED FROM EMBRYONIC STEM CELLS

MILAZI S, PRICE A, BROOKS BR, MOUGEOT J-L

Carolinas Medical Center, Department of Neurology, Neuromuscular/ALS Research Laboratory, Charlotte, NC, United States

E-mail address for correspondence: stephanie.milazi@gmail.com

Keywords: ES Cells, motor neurons, enrichment

Background: The etiology of Amyotrophic Lateral Sclerosis (ALS) is still poorly understood. There is no cure and only one FDA approved drug, riluzole, is known to extend the lifespan of ALS patients by a few months on average. Several groups have produced MNs derived from embryonic stem

cells (ESCs) and induced pluripotent stem cells (iPS) from ALS patients to study ALS and develop new therapies. One limitation of isolating pure MNs is that, following induced differentiation, undifferentiated cells are still present in embryoid bodies (EBs) that contain MNs. EBs are aggregates of cells that are difficult to dissociate into single cell suspension without affecting MN viability. Undifferentiated cells may interfere with the interpretation of the results in drug screening assays that affect the survival of MNs and therefore render understanding of ALS-specific cell death mechanisms more difficult. We have developed a method based on step-gradient centrifugation to isolate viable MNs from dissociated EBs.

Objective: To isolate and subculture differentiated motor neurons derived from mouse embryonic stem cells within embryoid bodies for the development of drug screening assays and transplantation therapies.

Methods: Mouse ESCs (HBG3:GFP:HB9) were differentiated into MNs using a modified protocol by Wichterle *et al.* (2008). EBs containing GFP-positive MNs and undifferentiated cells were gently dissociated into single cells by enzymatic and chemical treatments without mechanical dissociation. The single cell suspension was layered on Optiprep^{TM} medium step-gradient ranging from 8-20%. To determine purity, we used FACS to quantify GFP-positive cells corresponding to MNs.

Results: We were able to consistently produce EBs which in total contained over 68%±8 (mean±std dev) of MNs when dissociated and analyzed by FACS. After dissociation, the single cell suspension was co-cultured onto myoblast cells (C2C12) and neuromuscular junction formation was verified by immunohistochemistry using alpha-bungarotoxin conjugate which binds to acetylcholine receptor. In addition, we were able to obtain MN fractions enriched to 88%±6 (mean±std dev) by step-gradient centrifugation.

Discussion and conclusion: The development of standards for MNs addressing physiological relevance such as the phenotype (neurite outgrowth), and the ability of MNs to form neuromuscular junctions with muscles is necessary to design an appropriate assay. Our data supports dissociation without trituration and step-gradient centrifugation can be used to enrich ES cell-derived MNs retaining high viability, without the use of a cell sorter or the extensive use of antibodies for negative or positive selection. This study provides data for developing criteria for using MNs enriched by gradient centrifugation for cell-based assays for preclinical drug screening and cell transplantation therapies in ALS and other motor neuron disorders.

P47 DEVELOPMENT OF *IN VITRO* HUMAN NEUROMUSCULAR JUNCTION SYSTEMS

GUO X[1], GONZALEZ M[1,2], DAS M[3], RUMSEY J[4], STANCESCU M[1], JOHE K[5], DAVIS H[1,2], MOLNAR P[1], HICKMAN J[1,2]

[1]*Nanoscience Technology Center,* [2]*Biomolecular Science Center, Burnett School of Biomedical Sciences; University of Central Florida, Orlando, FL, United States,* [3]*Bioscience and Bioengineering, Indian Institute of Technology, Kanpur (UP), India,* [4]*Sanford-Burnham Medical Research Institute, Lake Nona, FL, United States,* [5]*Neuralstem, Inc., Rockville, Maryland, United States*

E-mail address for correspondence: xguo@mail.ucf.edu

Keywords: human, in vitro, neuromuscular junction

Background: To date, studies concerning motoneuron diseases (MNDs) have been primarily carried out in animal models or postmortem human tissues. However, animal models do not recreate the full process of human diseases, emphasizing a need for human-based systems. Conversely, studies from postmortem human tissues usually only capture late stage pathology rather than the real cause which occur at early stages. In addition, the effectiveness and toxicity of therapeutic drugs on human cells/systems are generally not assessed until preclinical studies, which are under strict regulations and expensive and time consuming. Therefore, human stem cell-based *in vitro* model systems are necessary for the study of MNDs and their therapies.

Objectives: In this study, we strived to build an *in vitro* human-based Neuromuscular Junction (NMJ) system to address this need.

Methods: Due to the limited availability of primary human tissue, human stem cell-derived motoneurons and myoblasts were utilized, in addition to rat embryonic skeletal muscle. NMJs were analyzed by phase microscopy, immunocytochemistry with confocal microscopy, and functional assays.

Result: First, motoneurons were differentiated from the human spinal cord stem cell line NSI-566RSC (1). A serum-free media was developed and the cells were cultured on a synthetic non-biological surface. These motoneurons were functionally mature based on the analysis with immunocytochemistry and electrophysiology. Then, the motoneurons were co-cultured with rat embryonic skeletal muscle to test their capability to form chimeric, functional NMJs. Based on the immunocytochemical analysis and the functional assays, successful NMJs were formed between human motoneurons and rat myotubes (2). These motoneurons were also co-cultured with myotubes developed from human skeletal muscle stem cells in a similar defined system. NMJ formation was demonstrated by the co-localization of motoneuron terminals and Acetylcholine (Ach) receptor clusters on the myotubes. Particularly, the observation of muscle contractions that could be ended by the Ach receptor antagonist, curare, confirmed the formation of functional NMJs.

Discussion and conclusions: The cross-species NMJ formation between human motoneurons and rat skeletal muscles not only uncovers the essential elements for NMJ formation shared in both human and rat, but also provides scientific basis for stem cell replacement studies which are typically conducted in rats. Due to their defined nature, these human-based systems, especially the human motoneuron-human skeletal muscle pairing, can be easily dissected and manipulated to study human NMJ formation, maintenance and repair, to investigate the mechanism of MND, and to perform high throughput drug screening. In summary, these human-based *in vitro* model systems would provide a novel avenue for the study and therapeutic investigation of MND.

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P48 COMPARTMENTALISED EXCITOTOXICITY IN PRIMARY CULTURED NEURONS: A NOVEL *IN VITRO* MODEL OF ALS PATHOLOGY

HOSIE KA[1,2], KING AE[1,2], YAP YC[1,2], BLIZZARD CA[1,2], VICKERS JC[1,2], DICKSON TC[1,2]

[1]*Menzies Research Institute, Hobart, Tasmania, Australia,* [2]*University of Tasmania, Hobart, Tasmania, Australia*

E-mail address for correspondence: khosie@utas.edu.au

Keywords: excitotoxicity, neuromuscular junction, axon degeneration

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by distal axon degeneration prior to symptoms. We have linked glutamate excitotoxicity in primary motoneurons to ALS-like distal axon degeneration. It currently remains unclear if toxicity is directed through the distal axon at the neuromuscular junction, or the somatodendritic compartment within the spinal cord.

Objectives: The aim of this study is to investigate the hypothesis that 'somatodendritic excitotoxin exposure can result in primary degeneration of the distal axon'. We are investigating this hypothesis in a compartmentalized primary culture model involving growth of primary spinal motor neuron cell bodies on a feeder layer of glial cells and distal axons extending to cultured skeletal muscle, allowing formation of a neuromuscular synaptic structure.

Methods: Primary cortical and spinal motoneurons are derived from E15 and E13 embryonic rodents respectively. Primary glia and skeletal muscle cultures are derived from neonatal rodent pups. Primary cortical neurons are grown as monocultures in compartmentalized microfluidic chambers (Xona) that allow separation of somatodendritic and axonal compartments. Spinal motoneurons are grown on feeder layers of glial cells or skeletal muscle or in microfluidic chambers with glial and muscle cultures as described above. Control and treated cultures are analysed using standard immunocytochemical methods.

Results: Cortical neurons grown in microfluidic chambers demonstrated separation of axonal and somatodendritic compartments as evidenced by MAP2 immunoreactivity (dendrites) confined to the somal chamber, and NFM immunoreactivity (axons) present in the axonal chamber. Skeletal myoblast cultures matured into contractile, multinucleated myotubes, with rudimentary neuromuscular junctions evident when co-cultured with spinal motoneurons. Furthermore spinal motoneurons cultured on muscle exhibited significantly ($P<0.05$) diminished survival and altered morphology, with fewer dendrites ($n=5$, 2.6 ± 0.3) and significantly longer axons ($n=5$, $1363\ \mu m\pm104$) compared to motoneurons co-cultured with astrocytes (dendrite number: $n=4$, 4.5 ± 0.8, axon length: $n=5$, $759\ \mu m\pm105$). To investigate the hypothesis that excitotoxicity mediated through the somatodendritic compartment results in distal axon degeneration, cultured cortical neurons (14 days *in vitro*) in microfluidic chambers were compartmentally exposed to 100 μM glutamate.
Excitotoxin exposure to the somatodendritic compartment resulted in axonal blebbing and fragmentation within both compartments, with increased overall distal axon damage (preliminary data). Somatodendritic excitotoxin exposure resulted in dendritic beading and a significant ($P<0.05$) increase in apoptotic nuclei ($n=4$, $64\%\pm4.5$) relative to untreated controls ($n=4$, $40\%\pm4.4$). Axonal excitotoxin exposure resulted in increased axonal fragmentation relative to control chambers, decreased relative to somatodendritic treated chambers (preliminary data). Future experiments will investigate mechanisms of site-specific excitotoxicity in the spinal motoneuron model.

Discussion and conclusion: These data indicate that microfluidic chambers can be used to investigate the role of different neuronal compartments axon degeneration and may provide insights in mechanisms of degeneration in ALS.

P49 EPIGALLOCATECHIN-3-GALLATE PROTECTS MOTOR NEURONS AND REGULATES GLUTAMATE LEVEL

LIU Y, YU J, GUO Y, JIA Y, DUAN W, LI Z, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: motor neuron, glutamate excitotoxicity, EGCG

Background: Amyotrophic lateral sclerosis (ALS) is a progressive and lethal neurodegenerative disease, characterized by degeneration of motor neurons from cortex, brainstem and spinal cord. In ALS, glutamate-mediated neurotoxicity was first suggested as a mechanism of motor neuron death. Increased levels of glutamate were detected in the cerebrospinal fluids of 40% sporadic ALS patients. THA-induced glutamate excitotoxicity in organotypic spinal cord cultures has been one of the widely used models of motor neuron degeneration and has also been applied for development of neuroprotective strategies.

Objectives: To investigate the protective effects of EGCG on the glutamate excitotoxicity induced motor neuron injury.

Methods: Organotypic spinal cord cultures were prepared from lumbar spinal cord explants of 7-day-old SD rat pups. The cultures were divided into four groups at random: control, THA, EGCG+THA, NAC+THA (EGCG or NAC pretreated 48 h then treated with EGCG+THA or NAC+THA). The number of motor neurons was assessed by immunohistochemistry, and glutamate concentrations in the culture medium and lipid peroxidation in spinal cord explants were measured using glutamate detection and TBARS assay kits, respectively. EAAT2 expression was measured by Western blot.

Results: Both 5 μM EGCG and 100 μM NAC blocked THA-induced motor neuron death and decreased TBARS levels. Different from NAC, protection of motor neurons by 5 μM EGCG is associated with regulating the glutamate level in the culture medium. However, there was no change of EAAT2 expression after treatment with 5 μM EGCG for 48 h. This property of EGCG may be not due to its intrinsic antioxidative activity, because another antioxidant NAC could not regulate glutamate levels under the same condition.

Discussion: In the present study, we found that motor neuron protection by EGCG was accompanied by regulation of glutamate levels in the synaptic cleft, and there have been few studies investigating this mechanism of EGCG. We speculate that EGCG could increase the activity of EAAT2 and the glutamate uptake of astrocytes, so the medium glutamate levels decreased after treatment with EGCG for 3 weeks. In view of the importance of glutamate excitotoxicity in ALS, EGCG may be a potential candidate for ALS therapy.

Conclusions: EGCG can regulate glutamate levels and inhibit lipid peroxidation and protect motor neurons against THA-induced toxicity.

P50 PROTECTIVE EFFECT OF COMBINATION OF SULFORAPHANE AND RILUZOLE ON GLUTAMATE-MEDIATED EXCITOTOXICITY

LI C, CHANG G, GUO Y, JIA Y, LIU Y, SONG X

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: excitotoxicity, sulforaphane, riluzole

Background: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease characterized by progressive and selective death of motor neurons. Evidences suggest that glutamate-induced excitotoxicity is an important pathogenic factor.

Objectives: To investigate whether the combination of sulforaphane (SF) and riluzole was more effective than either used alone in the protection against glutamate-mediated excitotoxicity.

Methods: Threohydroxyaspartate (THA) treated organotypic spinal cord cultures was used as a selective motor neuron injury model. Five groups were included: control group, THA group (100 μM), riluzole treatment group (5 μM or 2 μM), SF treatment group (10 μM or 4 μM) and SF-riluzole combined treatment group. Explants from each group were harvested for immunohistochemical staining with anti-neurofilament (SMI-32) antibody. Expression of erythroid 2-related factor 2 (Nrf2), NADPH: quinone oxidoreductase 1 (NQO1) and heme oxygenase 1(HO-1) were measured by Western blot analysis. Levels of LDH, MDA and glutamate in the culture medium were measured.

Results: In our study, SF-riluzole combined treatment not only stimulated the expression of Nrf2, NQO1 and HO-1, but also reduced the extracellular accumulation of glutamate. When used at optimal doses, SF (10 μM) and riluzole (5 μM), either alone or in combination, all exerted significant and similar neuroprotection, as measured by the number of motor neurons, medium MDA and LDH level. When used at lower doses, SF (4 μM) and riluzole (2 μM), the combined treatment group was better than either used alone.

Discussion: The results presented in this study strongly suggest that the combination of SF and riluzole at lower doses was more effective than either used alone in the protection against glutamate-mediated excitotoxicity. Such a combination has never been studied before. It represents a novel approach in the potential cocktail therapy, as riluzole primarily modulates glutamate signaling, whereas sulforaphane modulates antioxidative functions.

Conclusions: SF and riluzole combined treatment may represent a potential therapy against excitotoxicity induced motor neuron injury.

P51 PROTECTIVE EFFECTS OF RESVERATROL ON AN ALS CELL CULTURE MODEL

WANG J, ZHANG Y, FAN D, DENG M

Peking University Third Hospital, Beijing, China

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: VSC4.1 cell line, hSOD1G93A, resveratrol

Background and objectives: Resveratrol has recently been widely reported as an age delaying and neuroprotective compound, and it seems to exert benefit by a mimicking calorie restriction effect and activating SIRT1. In *in vivo* and *in vitro* studies of amyotrophic lateral sclerosis (ALS), the effect of calorie restriction and SIRT1 activation is controversial.

Methods: In the present study, we constructed an ALS *in vitro* cell culture model by stably expressing human superoxide dismutase 1(hSOD1)^{wt} and mutant hSOD1^{G93A} in a motor neuron like VSC4.1 cell line, which expressed matured motor neuron specific marker HB9 after differentiation. Then, we investigated the effect of resveratrol on this cell culture model.

Results: During a 24~48 hour course, we found that 0.5~50 μM resveratrol showed dose-dependent protective effects on the hSOD1^{G93A} bearing ALS cell culture model, by increasing cell viability, promoting neurite outgrowth, preventing cell apoptosis course and elevating cellular ATP level. We also showed in our study that this effect was at least partly achieved by accelerating mitochondrial biosynthesis, as resveratrol remarkably increased the mRNA level of PGC1-α and mitofusin 2 in the hSOD1^{G93A} bearing ALS cell model 24~48 hours after treatment, which was prevented by the SIRT1 inhibitor nicotinamide. Meanwhile, the ability of resveratrol to promote neurite outgrowth could not be blocked by SIRT1 inhibition, which indicated that resveratol might affect another SIRT1 independent pathway to exert benefit on the hSOD1^{G93A} bearing ALS cell model.

Conclusions: Our results suggest that resveratrol protects hSOD1^{G93A} bearing ALS cell culture model from mutant SOD1-mediated motor neuron cell toxicity partially by activation of SIRT1, which may be a potent therapeutic target for preventing the motor neuron degeneration of ALS.

P52 MUTANT TDP-43 INDUCED OXIDATIVE INJURY IN A MOTOR NEURON-LIKE CELL LINE

DUAN W, GUO Y, YU X, JIANG H, LU J, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: oxidative stress, HO-1, Nrf2

Background: Since the transactive response DNA-binding protein 43 (TDP-43) positive inclusions were detected in patients with frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-Us) and ALS, various missense mutations have been identified in this protein. TDP-43 is a nuclear protein which regulates transcription and RNA splicing, and participates in neurofilament mRNA transport and stability. To date, oxidative stress is regarded as an important pathway for the selective degeneration of motor neurons. In the present study, we hypothesized that mutant TDP-43 could mediate oxidative stress.

Objectives: To explore whether mutant TDP-43 could induce mitochondrial dysfunction and oxidative damage in the NSC34 cell line and whether sulforaphane could protect the cell line from the toxicity of mutant TDP-43.

Methods: Stable wild type and mutant TDP-43 (Q331K and M337V) transfected NSC34 cell lines were established; Western blot, quantitative real time PCR, confocal microscopy; and immunocytochemistry analysis were used. Cell proliferation was measured by Cell Counting Kit-8. Mitochondria transmembrane potential was measured by flow cytometry. TBARS in the cells and LDH content in the medium were tested.

Results: Mutant TDP-43 induced mitochondrial dysfunction, oxidative damage and nuclear accumulation of nuclear factor

E2-related factor 2 (Nrf2). HO-1 was down-regulated in cells expressing the mutant TDP-43, and could not be restored by sulforaphane. Nevertheless, sulforaphane reduced the level of lactate dehydrogenase and lipoperoxidation products in cells expressing mutant TDP-43. However, sulforaphane could up-regulate the expression of HO-1 and NAD(P)H/quinone oxidoreductase-1 (NQO-1) in cells transfected with empty vector and the wild-type TDP-43.

Discussion: We found that mutant TDP-43 induced mitochondrial injury and oxidative damage, especially TDP-43 Q331K. The similar changes of mitochondrial dysfunction and oxidative damage in motor neurons have been reported in patients who suffered from sporadic or familiar ALS. Therefore, motor neuron-like cell line expressing mutant TDP-43 mimics the pathological changes of motor neurons *in vivo*. Activating Nrf2 is a new and effective therapeutic strategy for ALS. Antioxidant defense elevated by activating Nrf2 can protect motor neurons from oxidative damage and apoptosis. Subsequently, sulforaphane protected cells against mutant TDP-43 independent of Nrf2-ARE pathway, nevertheless it had an action on the empty and wild-type TDP-43 cell lines by activating Nrf2 and up-regulating the expression of HO-1 and NQO-1 in a dose-dependent manner.

Conclusions: Mutant TDP-43 results in mitochondrial dysfunction, oxidative injury and reduced HO-1 expression in NSC34 cell lines.

P53 MG132 PROMOTES NEURITE OUTGROWTH INHIBITED BY MUTANT TAR DNA-BINDING PROTEIN-43 (TDP-43) VIA ACTIVATING HEME OXYGENASE-1(HO-1)

DUAN W, GUO Y, JIANG H, YU X, LU J, LI Z, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

E-mail address for correspondence: chunyanli5@yahoo.com.cn

Keywords: TDP-43, HO-1, Nrf2

Background: TDP-43 is a nuclear protein which regulates transcription and RNA splicing, and participates in neurofilament mRNA transport and stability. Recently, we established wild type and mutant TDP-43 (Q331K and M337V) cell lines. Furthermore, we observed that mutant TDP-43 had an adverse effect on neurite outgrowth in stable cell lines.

Objectives: To explore the effect of MG132 on the mutant TDP-43 cell lines.

Methods: Stable wild type and mutant TDP-43 (Q331K and M337V) transfected NSC34 cell lines were established, and the length of neurites were measured by FV10-SVW 1.7 viewer. Western blot, lipoperoxidation and LDH analysis were used.

Results: The neurites of NSC34 cell line expressing mutant TDP-43, especially TDP-43 Q331K, were significantly shorter than that expressing WT TDP-43. Lower doses (0.1 and 1 μM) of MG132 didn't increase mutant TDP-43 expression or LDH level in the cells transfected mutant TDP-43. However, 5 μM MG132 significantly induced cellular damage. Lower dose inhibition of the activity of proteasome promoted the neurite outgrowth in the mutant TDP-43 expressing cells. In our previous studies, mutant TDP-43 down-regulated the expression of HO-1. So, we speculate whether lower dose MG132 could restore the expression of HO-1. Indeed, HO-1 was restored by MG132. The transcription factor Nrf2 regulates the endogenous antioxidative capacity through transactivating phase-II detoxification genes, known as HO-1. MG132 induced the accumulation of Nrf2 into the nucleus in cells stably transfected WT TDP-43 in a dose-dependent manner, however reduced the accumulation of Nrf2 in the nucleus in mutant TDP-43 expressing cells.

Discussion: We found that mutant TDP-43 inhibited the neurite outgrowth. The shorter neurites in the cells stably transfected with mutant TDP-43 could suggest that mutant TDP-43 lost its normal function and gained toxicity. It is well known that inhibiting UPS by both pharmacological and genetic means delays axon degeneration and promotes neurite outgrowth. Indeed, we found that MG132 promoted the neurite outgrowth in the cells stably transfected with mutant TDP-43 as well as wild type TDP-43. In ALS, spinal motor neurons demonstrate ubiquitin immunopositive cytoplasmic inclusions which are also immunopositive for TDP-43. Its presence in ubiquitin positive inclusions strongly indicated TDP-43 was at least regulated by the UPS. We also found that non-toxic MG132 up-regulated HO1 expression in a dose-dependent manner and increased the antioxidative capacity accompanied with stimulating neurite outgrowth.

Conclusions: Mutant TDP-43 inhibits neurite outgrowth and lower dose MG132 stimulates neurite outgrowth. This action of MG132 could be associated with increase of HO1 expression.

P54 ENDOPLASMIC RETICULUM STRESS IS LINKED WITH TDP-43 REDISTRIBUTION IN ALS

WALKER A[1,2], MAY[2], TURNER B[2], PARISH C[2], SOO KY[1], FARG M[1], HORNE M[2,3], ATKIN J[1,2]

[1]*Department of Biochemistry, La Trobe University, Bundoora, Victoria, Australia,* [2]*Howard Florey Institute, Florey Neuroscience Institutes, Parkville, Victoria, Australia,* [3]*Department of Neurology, St Vincent's Hospital, Fitzroy, Victoria, Australia*

E-mail address for correspondence: adam.walker@latrobe.edu.au

Keywords: ER stress, TDP-43, redistribution

Background: Endoplasmic reticulum (ER) stress results from a variety of cellular insults, and leads to activation of signalling pathways known as the unfolded protein response (UPR). The UPR can alleviate ER stress by increasing chaperone production, inhibiting general protein translation and enhancing protein degradation. However if ER stress is prolonged, as in ALS, then cell death is triggered. ER stress occurs prior to symptom onset in SOD1G93A mice, and genetic ablation of UPR proteins delays disease onset and increases survival of these mice, suggesting an important role for ER stress in pathogenesis. Recently, ER stress was also identified in sporadic ALS patients. TDP-43 is the major constituent of inclusions in most ALS patients, and altered TDP-43 protein processing and distribution is a key feature of disease. Mutations to the gene encoding TDP-43 are also linked with familial and sporadic forms of ALS.

Objectives: The aims of this study were to investigate whether ER stress leads to altered cellular distribution or aggregation of TDP-43, and whether TDP-43 expression causes UPR induction in cell culture.

Methods: Plasmids encoding full-length or C-terminal fragment TDP-43 were constructed. Neuroblastoma cells and primary rodent cortical neurons were transfected, and TDP-43 expression, sub-cellular location and solubility were monitored using immunocytochemistry, confocal microscopy and immunoblotting. ER stress was monitored using immunocytochemistry and immunoblotting for UPR markers.

Results: The majority of cells expressing either full-length wildtype or six different ALS-linked mutants had primarily nuclear localised TDP-43 while the C-terminal fragment proteins were located throughout the cytoplasm. Proteasome inhibition caused a dramatic increase in the proportion of cells with cytoplasmic localisation of TDP-43 and resulted in the formation of inclusion-like structures, particularly when the C-terminal TDP-43 fragments were expressed. Pharmacologically-induced ER stress also caused TDP-43 redistribution, and cells expressing mutant TDP-43 showed increased UPR induction compared to controls.

Discussion and conclusions: These findings indicate that ER stress is a feature of mutant TDP-43 linked ALS, and that UPR induction results in redistribution of TDP-43 from the nucleus to the cytoplasm in a manner reminiscent of disease pathology. ER stress could therefore be an early triggering event in ALS, and therapeutic targeting of the UPR could be beneficial in treatment of both familial and sporadic forms of disease.

P55 METALS AND OXIDATIVE STRESS INDUCE MISLOCALIZATION AND AGGREGATION OF ENDOGENOUS TDP-43

MEYEROWITZ J[1,2], PARKER S[1,2], CARAGOUNIS A[1,2], PRICE K[1,2], SOON C[1,2], FILIZ G[1,2], MASTERS C[3], LI Q-X[1], CROUCH P[1,2], WHITE A[1,2]

[1]*Department of Pathology,* [2]*Centre for Neuroscience; The University of Melbourne, Victoria, Australia,* [3]*Mental Health Research Institute, Victoria, Australia*

E-mail address for correspondence: arwhite@unimelb.edu.au

Keywords: TDP-43, oxidative stress, metals

Background: Neuronal aggregates containing ubiquitinated and phosphorylated TDP-43 are pathological hallmarks in the spectrum of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Changes to TDP-43 metabolism have also been reported in other neurodegenerative diseases. In affected neurons, TDP-43 undergoes C-terminal fragmentation, phosphorylation and ubiquitination and forms aggregates in the cytoplasm or nucleus. Loss of nuclear TDP-43 expression is also prominent. While *in vitro* studies have been able to re-capitulate many of these features using transfected cell culture models, little is known about the biochemical mechanisms that underlie pathological changes to endogenous TDP-43.

Objective: As altered metal ion homeostasis and oxidative stress are central features of neurodegeneration, including FTD and ALS, we sought to determine the effects of these factors on endogenous TDP-43 metabolism in mammalian cells.

Methods: Cultures of neuronal-like SY5Y cells were treated with biometals or subjected to mild oxidative insults and changes to TDP-43 metabolism were examined by immunoblot and immunofluorescence.

Results: Treatment of SY5Y cells expressing endogenous TDP-43 with zinc (Zn) induced depletion of nuclear TDP-43 expression and formation of inclusions in the nucleus and cytoplasm that were TDP-43 positive. No evidence of C-terminal fragmentation, phosphorylation or ubiquitination was observed. The depletion and aggregation of TDP-43 was associated with the specific action of Zn and was not seen with copper or iron. Exposure of cells to specific modulators of oxidative stress induced depletion of nuclear TDP-43, increased diffuse cytoplasmic TDP-43 localization, elevated levels of a 35 kDa C-terminal fragment and led to formation of cytoplasmic ubiquitin-positive inclusions containing TDP-43. Inhibition of caspase activation partially abrogated cytoplasmic inclusion formation concomitant with reduced expression of the 35 kDa C-terminal fragment.

Conclusions: These findings suggest that altered metal homeostasis or oxidative stress, which are common features of neurodegeneration, may affect metabolism of endogenous neuronal TDP-43 resulting in accumulation in cytoplasmic inclusions.

P56 MODE OF DEATH AND ROLE OF ROS IN HOMOCYSTEINE (HCY) AND AAPH (2,2'-AZOBIS-2-METHYL-PROPANIMIDAMIDE)-INDUCED CELL DEATH IN DIFFERENTIATED MOTOR NEURON - NEUROBLASTOMA HYBRID NSC-34D CELLS

HEMENDINGER R, ARMSTRONG III E, BROOKS BR

Carolinas Medical Center, Charlotte, NC, United States

E-mail address for correspondence: rhemendinger@carolinas.org

Keywords: oxidative stress, cell death mechanisms, NSC-34 cells

Background: Oxidative stress has been implicated as an early step in the pathogenesis of ALS and animal models of ALS. Hydrogen peroxide is commonly used *in vitro* to study oxidative stress. Our previous work with the antioxidants, luteolin and huperzine A, demonstrated both neuroprotection and neurorescue against hydrogen peroxide (H_2O_2)-induced cell death in an *in vitro* model of sporadic ALS (1,2). As H_2O_2 is a general oxidative stressor, we studied a free radical generator capable of generating peroxyl radicals and molecular nitrogen, AAPH.

Objective: The current study examines the toxicity of AAPH in our *in vitro* model of sporadic ALS in comparison to $H_2O_2$and Hcy (a compound shown to induce oxidative stress in other cell types).

Methods: The toxicity of AAPH was examined in differentiated motor neuron-neuroblastoma hybrid NSC-34 (NSC-34_D) cells. Cell death was assessed after a 24 h exposure to AAPH doses ranging from 0 mM to 5 mM. Cell death was determined using nuclear staining with Hoechst 33342 and propidium iodide and a CellQuanti assay for metabolic activity at 24 h of exposure. Additional assays investigating the dose-response induction of ROS production during a 1 h exposure to AAPH and homocysteine were performed. Analysis of variance and Fisher's LSD test were used to anlyze the data at a P value of 0.05.

Results: Cell death increased in a dose-dependent manner with AAPH, reaching 46% at 3.0 mM and increasing to 84% at 5.0 mM ($P<0.05$). The mode of cell death was primarily via necrosis with AAPH. In contrast, Hcy induced primarily apoptotic cell death (3). Metabolic activity assays demonstrated a significant dose-dependent reduction in metabolic activity at 24 h with AAPH ($P<0.05$) while Hcy had no effect on metabolic activity. Preliminary studies examining the role of ROS in the cell death induced by these agents were performed. A dose-response induction of ROS during a 1 h exposure to AAPH was observed in NSC-34_D cells and in the control heptocarcinoma cell line, HepG2. In contrast, homocysteine failed to induce ROS up to 500 mM dose in this assay with either cell line.

Conclusions: AAPH induces necrotic cell death and ROS generation in a dose-dependent fashion in the differentiated motor neuron-neuroblastoma hybrid NSC-34_D cells. In contrast, Hcy, known to play a role in the pathogenesis of ALS and cause oxidative stress in endothelial cells, induces apoptotic cell death in these NSC-34_D cells without significant ROS generation following a short-term exposure. Understanding how AAPH and Hcy modulate molecular

targets will allow us to understand the processes involved in neuronal cell death in our *in vitro* model and in patients with ALS.

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P57 PROTEIN DISULPHIDE ISOMERASE REGULATES SOD1 ACTIVITY AND CONTROLS CYTOCHROME C-CATALYZED PEROXIDATION – IMPLICATIONS FOR MITOCHONDRIAL ROS PRODUCTION IN ALS MODELS

JARONEN M[1], AHTONIEMI T[2], KOISTINAHO J[1], GOLDSTEINS G[1]

[1]*A. I. Virtanen Institute for Molecular Sciences University of Eastern Finland, Kuopio, Finland,* [2]*Medeia Therapeutics Ltd, Kuopio, Finland*

E-mail address for correspondence: merja.jaronen@uef.fi

Keywords: SOD1, protein disulfide isomerase, ROS

Background: Increased mitochondrial production of reactive oxygen species (ROS) has been implicated in a number of neurodegenerative diseases, including ALS. Some familial forms of ALS are associated with point mutations in the superoxide dismutase 1 (SOD1) gene. Nevertheless, the mechanism of the gain of toxic function remains unknown.
SOD1, an abundant cytosolic enzyme, is present in an inactive form also in the mitochondrial intermembrane space (IMS). SOD1 activity in this compartment is controlled by the redox state of an intramolecular disulphide bond. The formation of this bond and activation of SOD1 is executed by protein disulfide isomerase (PDI), which has been found also in mitochondria.
We have previously demonstrated that in G93A-SOD1 rats the mutant SOD1 is up-regulated in the IMS and possesses increased ability to bind the inner membrane of isolated mitoplasts. In parallel, we have shown that SOD1 activity in the IMS increases mitochondrial ROS production by enhancing hydroperoxide production, eventually resulting in augmented cytochrome c-catalyzed peroxidation. Finally, we have been able to demonstrate that PDI expression peaks in the spinal cord of a G93A-SOD1 rat model as well as in a mouse model at presymptomatic stage of the disease.

Objectives: Our previous findings allowed us to hypothesize that increased PDI expression leads to the aberrant control of SOD1 activity in the mitochondrial IMS and causes increased hydroperoxide production in this compartment, eventually resulting in neuronal vulnerability. In the current study our aim was to investigate whether PDI can exercise redox control of SOD1 activity leading to increased hydroperoxide production and cytochrome c-catalysed peroxidation *in vitro* and HEK-293 cell culture.

Methods: *In vitro* superoxide production was generated by xanthine/xanthine oxidase. Cytochrome c-catalyzed peroxidation was measured with dichlorodihydrofluorescein (DCF), a specific substrate for hydroperoxide, both *in vitro* and in HEK-293 cell culture. In cell culture model superoxide production was induced with paraquat.

Results: Our results show that PDI catalyzes reactivation of SOD1 after inactivation by disulphide bond reduction. This reactivation resulted in increased hydroperoxide production and cytochrome c-catalysed peroxidation. Both reactivation and increased peroxidation were inhibited by bacitracin, a PDI inhibitor. Inhibition of PDI by bacitracin suppressed also paraquat-induced hydroperoxide production in HEK-293 cells.

Discussion and conclusions: These results elucidate the possible role of PDI in controlling SOD1 activity within the IMS and its impact on mitochondrial ROS production in ALS models.
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P58 EFFECTS OF PALMITOYL CARNITINE ON BRAIN, SPINAL CORD AND HEART MITOCHONDRIA FROM WILD TYPE AND TRANSGENIC SOD1 RATS

PANOV A, KUBALIK N, BROOKS BR

Carolinas Medical Center, Charlotte, North Carolina, United States

E-mail address for correspondence: sibiryak111@yahoo.com

Keywords: brain, spinal cord heart mitochondria, ROS

Background: Mitochondrial dysfunctions contribute to the loss of motor neurons in ALS. Several metabolites are oxidized simultaneously by mitochondria i*n vivo* (1). The higher vulnerability of motor neuron mitochondria may be associated with some metabolic features unique for central nervous system tissue. Brain and spinal cord mitochondria from SOD1 transgenic rats have substrate-specific increases in ROS production (2). Unlike heart, brain and spinal cord mitochondria do not oxidize fatty acids presumably due to low activity of 3-ketoacyl-coenzyme A thiolase.

Objectives: Do the tissue-specific metabolic preferences affect mitochondrial dysfunctions in ALS?

Methods: Heart, brain and spinal cord mitochondria were isolated from wild type and G93A SOD1 transgenic rats. Pyruvate, glutamate, malate, succinate, palmitoyl-carnitine and their mixtures were used as substrates. Respiratory activity was determined with a Clark electrode, and ROS generation was measured by the Amplex Red method.

Results: Heart mitochondria oxidized palmitoyl-carnitine or succinate alone very poorly in State 3, and respiration was inhibited upon uncoupling. However, when palmitoyl-carnitine was combined with any other mitochondrial substrate (pyruvate, glutamate, or succinate), the rates of oxidative phosphorylation and uncoupled respiration increased by 40 to 70%. Importantly, resting respiration increased 64% with glutamate and succinate. As a result, production of ROS increased 3-4 fold with these combinations of substrates. This trend was more pronounced in SOD1 transgenic rat heart mitochondria. Brain and spinal cord mitochondria did not oxidize palmitoyl-carnitine. However, when palmitoyl-carnitine was added to glutamate, pyruvate or succinate, the rates of ROS generation increased 4-fold as compared with glutamate and malonate. Malonate, an inhibitor of succinate oxidation, completely abolished increased ROS with Succinate and palmitoyl-carnitine, but not glutamate and palmitoyl-carnitine. The latter was inhibited by addition of uncoupler CCCP. This suggests that with glutamate and palmitoyl-carnitine the reverse electron transport was also involved in increased ROS production, but the electrons were fed to the membrane's pool of coenzyme Q by the FAD enzyme acyl CoA dehydrogenase. However, the exact mechanisms by which substrates and palmitoyl-carnitine facilitate ROS production remain obscure.

Conclusions: Rat heart mitochondria, brain and spinal cord mitochondria show strong substrate preferences for maximal rates of oxidative phosphorylation and ROS production. Palmitoyl carnitine significantly increased ROS production in all types of mitochondria. Although normally brain mitochondria do not oxidize palmitoyl-carnitine, its presence may facilitate oxidative stress in spinal cord of SOD1 transgenic rats.

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P59 GLIAL DYSFUNCTION IN ALS: REDUCED ACTIVITY OF THE MONOCARBOXYLATE TRANSPORTER, MCT1, PRODUCES CELL DEATH OF OVERACTIVE MOTOR NEURONS

MORRISON B, LI Y, TSINGALIA A, COCCIA C, ROTHSTEIN J

The Johns Hopkins University, Baltimore, Maryland, United States

E-mail address for correspondence: brainorris7@jhmi.edu

Keywords: metabolism, glutamate, astrocyte

Background: Several lines of evidence have suggested a role for astrocytes in the degeneration of motor neurons in amyotrophic lateral sclerosis (ALS). Astrocytes support the function of motor neurons through several mechanisms, including secretion of trophic factors, removal of glutamate from the synapse, and supplying the energy substrate lactate. This latter function is termed the astrocyte-neuron lactate shuttle. In this proposed shuttle, lactate produced by astrocytes through glycolysis is transported into the extracellular space through monocarboxylate transporters (MCTs) where it can be taken up by neurons using distinct MCTs, converted to pyruvate, and used within mitochondria to produce ATP. One of the primary astrocytic MCTs, MCT1, has been shown to be reduced in patients with ALS, and we propose that this contributes to motor neuron vulnerability by interrupting supply of lactate. Of course, motor neurons can also generate ATP from glucose; and therefore, we hypothesize that lactate is particularly critical for motor neurons when glucose supply is not sufficient to maintain neuronal metabolic activity.

Objectives: To investigate whether motor neurons *in vitro* and *in vivo* are more dependent on astrocyte-derived lactate when metabolically stressed by depolarization, overactivity, or injury.

Methods: To investigate this hypothesis *in vitro*, we exposed organotypic spinal cord cultures to three treatments expected to produce motor neuron depolarization and increased metabolic activity (ie, high potassium, the GABA antagonist bicuculline, and glutamate) in the presence or absence of a pharmacologic inhibitor to MCT1. *In vivo*, we used sciatic nerve crush as an injury paradigm, which is expected to increase the energy requirements of recovering motor neurons, in rats treated with intrathecal MCT1 inhibitor or vehicle alone.

Results: There was no cell death from the MCT1 inhibitor or any of the treatments that increase metabolic activity when added to the media alone. However, when the pharmacologic MCT1 inhibitor was combined with high potassium, bicuculline, or glutamate, there was a significant increase in cell death. Similarly *in vivo*, motor neurons in the lumbar spinal cord that had undergone nerve crush degenerated in rats treated with intrathecal MCT1 inhibitor, but not in those treated with vehicle alone.

Discussion: Elevated glutamate secondary to reduced activity of the astrocytic glutamate transporter, GLT1, has been shown to occur in ALS. In addition to potentially causing excitotoxic cell death, this elevated glutamate would lead to depolarization and hyperactivity of motor neurons. In combination with reduced expression of MCT1, our results from cell culture and animal models suggest that significant motor neuron cell loss would occur due to reduced lactate production from astrocytes and insufficient production of ATP to maintain critical neuronal functions.

Conclusion: Reduced levels of MCT1 in patients with ALS may contribute to motor neuron degeneration by increasing the vulnerability of overactive neurons.

P60 INSIGHTS INTO THE REGULATION OF ASTROCYTIC EAAT ACTIVITY FOR MOTOR NEURON PROTECTION

SHEEAN R[1,2], LAU C L[1,2], O'SHEA RD[1,2], BEART PM[1,2]

[1]*Florey Neuroscience Institute, Parkville, VIC, Australia,* [2]*University of Melbourne, Parkville, VIC, Australia*

E-mail address for correspondence: rebecca.sheean@florey.edu.au

Keywords: glutamate, transporters, pharmacology

Background: In Motor Neuron Disease (MND), glial cells strongly influence the demise of the motor neurons by contributing to inflammatory and excitotoxic mechanisms. Changes in astrocytic phenotype and cytoskeletal arrangement are key indicators of astrocytic reactivity. Astrocytes are responsible for the removal of the neurotransmitter L-glutamate (Glu) from the extracellular space to prevent neuronal injury and maintain synaptic signalling. Glu uptake is performed by excitatory amino acid transporters (EAATs), with the astrocytic EAATs, EAAT1 and EAAT2, responsible for the bulk of Glu uptake in brain. Loss of EAAT function is damaging to MNs, and increased EAAT2 expression is neuroprotective in MND models. The relationship between astrocytic morphology and EAAT function remains poorly understood, and understanding this interface may reveal how EAAT modulation can be exploited as a potential treatment in MND.

Objectives: To investigate the regulation of EAAT activity in astrocytes by altering astrocytic phenotype and EAAT distribution through pharmacological manipulation.

Methods: Primary cultures of mouse astrocytes were treated with rottlerin (a PKCδ inhibitor (100 mM, 6 h)) to reduce EAAT activity. Changes in astrocytic morphology and EAAT distribution were determined using immunocytochemistry, biotinylation and Western blotting. Glu uptake was investigated during and following rottlerin exposure. The rate of recovery of EAAT activity was examined during exposure to various drugs including monensin (a Na^+ ionophore (100 mM, 2 h) to explore the involvement of cellular mechanisms.

Results: Rottlerin treatment rapidly decreased [^{3}H]-d-aspartate uptake and caused cytoskeletal rearrangement. Biotinylation revealed increases in EAAT expression in all cellular fractions, including the cell surface with rottlerin treatment. After rottlerin removal, EAAT activity returned to control levels within 2-4 h. Monensin enhanced the recovery of [^{3}H]-d-aspartate uptake, suggesting that Na/K-ATPase may be attenuated by rottlerin treatment. This possibility is being investigated by measuring Rb+ uptake to determine effects of rottlerin and monensin on coupling to EAAT activity.

Discussion: Decreases in [^{3}H]-d-aspartate uptake are often associated with EAAT internalization or degradation.

Biotinylation revealed that both total and cell-surface expression of EAATs increased with rottlerin treatment, suggesting a homeostatic response. These results, plus the rapid rate of EAAT recovery following rottlerin removal, revealed this inhibition of EAAT function was not due to changes in protein expression or localization. The ability of monensin to increase the rate of recovery suggests the involvement of Na^+ in this EAAT inhibition. These data support the concept that EAATs are part of a molecular transport-complex, including the Na/K-ATPase.

Conclusion: EAAT activity and consequent homeostasis are essential for maintaining neuronal viability during injury. This study reveals that astrocytic EAAT expression is enhanced when EAAT activity is impaired, in an attempt to prevent injury. Additionally, our data suggest that maintenance of Na/K-ATPase activity is an integral component of this homeostatic response.

P61 USING LIVE CELL IMAGING IN A PRIMARY CULTURE MODEL OF ALS AS A METHOD TO DISCOVER THERAPEUTIC TARGETS

TRADEWELL M, COOPER L, DURHAM H

Montreal Neurological Institute, McGill University, Montreal, Canada

E-mail address for correspondence: miranda.tradewell@mcgill.ca

Keywords: calcium, mitochondria, aggregates

Background: Alterations in calcium homeostasis, mitochondrial function and protein aggregation have been identified as pathogenic mechanisms in models of ALS; however, how each of these mechanisms interact and fit together in a timeline has not been well-established. It is important to understand the primary and most important pathogenic events in order to properly target ALS therapies. Using genetically encoded and chemical fluorescent indicators, we have established a central role for the dysregulation of calcium homeostasis in motor neurons expressing mutant SOD1.

Methods: Mouse dissociated spinal cord cultures were prepared from embryonic mice as described previously (Durham *et al*, 1997), and plasmids encoding wild-type and mutant SOD1, TDP-43, and TLS-FUS were introduced by intranuclear microinjection. To measure mitochondria and ER calcium levels, we co-injected plasmids encoding mitochondrial ratiometric pericam or DER1 cameleon, respectively. Cytosolic calcium levels and mitochondrial membrane potential were measured using fura-2 and TMRM, respectively. Motor neurons were then imaged at several time points following microinjection.

Results: Measurement of calcium levels within different intracellular compartments indicated an early (day 1) elevation of mitochondrial calcium, which was accompanied by a loss in mitochondrial membrane potential and a rounding of their shape. This was followed by an elevation in ER calcium (day 3) and finally a rise in cytosolic calcium (day 5). Motor neurons with mutant SOD1 inclusions had even higher levels of cytosolic calcium, demonstrating a relationship between elevated calcium levels and inclusion formation. Expression of the calcium binding protein, calbindin D-28K, caused a reduction of cytosolic calcium and reduced mutant SOD1 inclusion formation whereas reducing inclusion formation by treatment with geldanamycin had no effect on calcium levels. Using the same imaging techniques in motor neurons, we have demonstrated that neither TDP-43 nor TLS-FUS mutants have a significant effect on mitochondrial shape; other parameters remain to be investigated.

Discussion and conclusions: Using live cell imaging techniques in primary motor neurons, we have established a timeline for calcium dysregulation in motor neurons expressing mutant SOD1. Calcium plays an early and central role in the demise of motor neurons. Using this model, we plan to examine the pathogenic events caused by the expression of other ALS-linked genes such as TDP-43 and TLS-FUS in order to find common disease mechanisms and potential therapeutic drug targets.

P62 CHRONOLOGICAL SEQUENCE OF EVENTS IN THE PATHOGENIC CASCADE IN ALS

FARG M[1], SOO K[1], WALKER A[2], TURNER B[2], HORNE M[2], ATKIN J[1,2]

[1]*Department of Biochemistry, La Trobe University, Bundoora, Australia,* [2]*Howard Florey Institute, University of Melbourne, Melbourne, Australia*

E-mail address for correspondence: julie.atkin@florey.edu.au

Keywords: disease mechanisms, protein misfolding, ER stress

Background: In ALS/MND, the pathology centres on central and peripheral motor neurons, and the severely damaged motor neurons observed at post mortem are the end stage of a pathophysiological cascade. Many mechanisms have been implicated in pathology, including ER stress, axonal transport, intracellular inclusion formation, and apoptosis, but their exact role in disease and the sequence in which they occur is unclear.

Objective: In this study, we aimed to identify the earliest events occurring in disease because these events are most likely to be involved in triggering the pathological cascade.

Methods: We used the motor neuron cell line, NSC-34, and neuronal Neuro2a cells transiently transfected with human wild-type and mutant SOD1 constructs tagged with EGFP. We also examined lumbar spinal cords obtained from transgenic $SOD1^{G93A}$ mice at pre-symptomatic (p10, p30, p60), symptom onset (p90) and disease end stage (p120).

Results and discussion: We have established that in motor neuron cell lines expression of SOD1 begins at 10 h. At t 14 hours we detected increased levels of ubiquitinated proteins, at 16 h ER stress is triggered and binding of mutant SOD1 to dynein and cellular transport proteins occurs. However the formation of mutant SOD1 oligomers, intracellular inclusions does not occur until 24 h post transfection. Interestingly, Bax recruitment occurred at 18 hours but apoptotic cell death was not triggered until after 24 h. In SOD1 mice, a physical interaction between mutant (but not wildtype SOD1) with proteins involved in intracellular transport was detected as early as postnatal age 10 days, 20 days prior to the onset of ER stress.

Conclusion: This study suggests that the first upstream events in the pathogenic cascasde triggered by mutant SOD1 are protein misfolding, perturbation of cellular transport and ER stress. The formation of mutant SOD1 intracellular inclusions and oligomers occurs relatively late in pathology, after the triggering of apoptosis by recruitment of Bax to mitochondria.

P63 MODELING ALS AXONAL PATHOLOGY *IN VITRO*

KING A, HOSIE K, BLIZZARD C, VICKERS J, DICKSON T

Menzies Research Institute, Hobart, Tasmania, Australia

E-mail address for correspondence: kingae@utas.edu.au

Keywords: axon pathology, excitotoxicity, glia

Background: Examination of ALS post-mortem tissue and mouse models of ALS reveals the presence of several types of pathological lesion and provides insight into the pathogenic mechanisms that underlie this devastating disease. Particularly prominent in mouse models of ALS from early time points is evidence of axon degeneration and dysfunction, including distal axon die back from the neuromuscular junction and more proximally within the axon, the presence of large swollen axon segments or spheroids.

Objectives: We have used primary cell culture techniques to investigate how pathological mechanisms implicated in ALS result in the types of pathology described. We have investigated how axonal pathology affects the function of the neuron, and in particular we are focused on determining the role of different neuronal compartments (somatodendritic or axonal) in the development of pathology. These data will be important for provision of therapeutic intervention in ALS.

Methods: Spinal motor neurons or cortical neurons were derived from embryonic rodents and grown for up to 21 days *in vitro* on glial feeder layers derived from neonatal rodents or Poly-L-lysine/laminin. To determine the role of neuronal compartments, cultures were grown in compartmentalized microfluidic chambers (Xona), which allow physical separation of the somatodendritic compartments from the axonal compartment for manipulation and analysis.

Results: In investigations to date we have been able to model two distinct types of axonal pathology that may have relevance to ALS. Spinal rat or mouse motor neurons, chronically exposed to kainic acid for up to 24 hours, developed bulbous swellings in the distal portion of their axons, which was associated with mislocalization of non-phosphorylated neurofilament protein to this region. This pathology typically progressed to cell death within 48 hours. Preliminary investigations using cortical neurons in compartmentalized chambers have supported the hypothesis that exposure of the somatodendritic compartment to excitotoxins can result in degeneration of the untreated axon segment. In other experiments we have demonstrated that spinal mouse motor neurons grown on a mixed glial feeder layer spontaneously developed swollen axon segments or spheroids filled with neurofilament proteins and organelles. These structures were not present when cells were grown in the absence of glial cells or in glia conditioned media. Strikingly, these spheroids were not associated with apoptosis or rapid cell death, but did involve abnormalities in axonal transport. The connection between distal and proximal axon pathology is the focus of ongoing investigation.

Discussion and conclusion: These data indicate that excitotoxicity can result in distal axon degeneration and that proximal axon spheroids can be the result of disturbed neuron-glia interaction. These primary cell culture models of axon pathology are useful tools for investigating mechanisms of axon degeneration and dysfunction in ALS.

P64 INVOLVEMENT OF THE MTOR SIGNALING IN AMYOTROPHIC LATERAL SCLEROSIS

ZONA C[1,2], PIERI M[1,2], CANU N[1,3], CARUNCHIO I[1,2], CAIOLI S[1,2]

[1]*University of Rome Tor Vergata, Department of Neuroscience, Rome, Italy,* [2]*Fondazione S. Lucia, Rome, Italy,* [3]*Institute of Neurobiology and Molecular Medicine, CNR, Rome, Italy*

E-mail address for correspondence: zona@uniroma2.it

Keywords: rapamycin, cortical neurons, electrophysiology

Background: Many papers have reported cortical hyperexcitability both in ALS patients and in the transgenic mouse model G93A of ALS. The involvement of ionic channels in the ALS pathology has been shown in G93A cortical neurons. Since hyperexcitability is an ALS feature, compounds able to reduce the neuronal excitability could be potential drugs useful in ALS clinical treatments. In this context, the mammalian target of rapamycin (mTOR) pathway has been shown to regulate ion channel synthesis and localization, thereby controlling neuronal excitability. In addition, mTOR is a key regulator of cell growth and proliferation and regulates autophagy. Interestingly, it has been reported that autophagy reduces mutant SOD1-mediated toxicity and that the induction of autophagy decreases mutant SOD1 protein levels.

Objectives: The aim of this work is to verify whether the excitability of G93A neurons is modified by rapamycin and whether the mTOR pathway is altered in G93A cortical neurons compared to controls.

Methods: G93A cortical neurons were used for patch clamp and Western blotting experiments. Current clamp cortical neuron recordings were performed and analysed, as previously reported. Proteins were visualized using antibodies to mTOR, PmTOR, Akt, Pakt, p70S6 and Pp70S6. Data are presented as mean + standard deviation (SD). Values of $P<0.05$ were considered statistically significant.

Results: To study the excitability of control non transgenic neurons and of G93A cortical neurons in control conditions and following rapamycin exposure (50 nM, 24 h), current steps from +40 pA to +200 pA were injected to elicit action potentials. In all tested cells, the inverse of the first interspike interval was taken as an estimate of the cell firing frequency. The treatment of G93A neurons with rapamycin significantly decreased the hyperexcitability ($P<0.02$) as far as control values. To verify whether in G93A neurons the decreased excitability induced by rapamycin was mediated by the mTOR pathway, we performed Western blotting analysis. We found that rapamycin was able to significantly reduce the level of mTOR phosphorylation both in G93A and in Control neurons. In addition, G93A neurons presented significantly higher levels of Pp70S6 compared to Control neurons, indicating the involvement of the mTOR signalling in ALS pathology because this level was reversed by rapamycin.

Discussion and conclusion: These results indicate the involvement of mTOR pathway in ALS pathology and that mTOR may regulate the electrical activity of the single neuron and mediate mechanisms of neuronal excitability. In conclusion, although our findings do not directly support a pathogenic mechanism of ALS, they point to the importance of the mTOR pathway in ALS pathology, potentially offering novel avenues for developing therapeutic strategies.

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P65 EFFECT OF ACYLATED STERYL GLUCOSIDES ON ALPHA-SYNUCLEIN AGGREGATION AND TOXICITY

USUKI S, MATSUO Y, KAMITANI T, YU RK

Medical College of Georgia, Augusta, Georgia, United States

E-mail address for correspondence: susuki@mcg.edu

Keywords: ALS-PDC, acylated steryl glucoside, alfa-synuclein

Background: The causal factors for sporadic or age-related neurological diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD) are still unknown, and disease progression shows involvement of genetic or environmental factors (1). The Guamanian variant of ALS-parkinsonism dementia complex (ALS-PDC) has received much attention, particularly with respect to its potential causes. These causes include its genetic origin, involvement of certain metals in drinking water, eg, low levels of calcium/magnesium and high levels of aluminum; the infectious agents cyanobacteria and *Helicobacter pylori,* traditional foods made from flying foxes, and consumption of flour made from cycad seed (*Cycas micronesica*) (2). It has been suggested that cycad seed may be an important factor in the pathogenesis of ALS-PDC as evidenced by a positive correlation between consumption of cycad seed and prevalence of the disease. Several compounds have been isolated from cycad seeds and are suspected to contribute to the motor neuron toxicity. Recently, β-N-methylamino-L-alanine (BMAA) and acylated steryl glucoside (ASG) have been reported to be principal components of cycad neurotoxin by a feeding experiment in mice.

Objectives: Acylated steryl glucosides (ASGs) are ubiquitously distributed in edible plant sources. To know whether edible plant-derived ASGs are a casual factor in ALS-PDC, soybean ASG (S-ASG), pre-germinated brown rice ASG (P-ASG), and *Helicobacter pylori*-derived ASG (H-ASG) were tested for an *in vitro* aggregation of α-synuclein and α-synuclein toxicity for yeast cells.

Methods: For α-synuclein aggregation reactions, human normal α-synuclein protein (80 μM) was incubated in a ATP regeneration system with 20 μg/mL of S-ASG, P-ASG and H-ASG, respectively. The aggregate formation was determined by a fluorescence of Thioflavin-T.
For the yeast-cell toxicity experiment, S-ASG, P-ASG and H-ASG were tested for cell growth on the plate of α-synuclein transformant and a control vector transformant of *Saccharomycces cerevisiae*.

Results: Compared with controls, S-ASG and P-ASG increased α-synuclein aggregation (1.4- and 1.3-fold higher, respectively), but H-ASG was not effective. For the yeast cell toxicity, α-synuclein transformants exhibited a growth defect on control plates as compared to a vector transformant. S-ASG and P-ASG had no effect on the vector transformant, but exhibited inhibition of cell growth on α-synuclein transformants.

Discussion and conclusions: The finding that S-ASG and P-ASG enhanced α-synuclein aggregation and toxicity suggests that ASGs represent a potential agent for disease development and progression, although these lipids are ubiquitously distributed in edible plant sources. S-ASG and P-ASG include a β-glucoside, but H-ASG includes an α-glucoside. The β-glucoside in the ASGs may be responsible for α-synuclein aggregation and toxicity.

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THEME 4 HUMAN CELL BIOLOGY AND PATHOLOGY

P66 GUAMANIAN NEURODEGENERATIVE DISEASE: ULTRASTRUCTURAL STUDIES OF SKIN

NOMURA M, ISHIKAWA H, YASUI K, WATANABE T, MIKAMI H, YAMANO T, SUZUKI M, ONO S

Teikyo University Chiba Medical Center, Ichihara/Chiba, Japan

E-mail address for correspondence: ono@med.teikyo-u.ac.jp

Keywords: ultrastructural studies, skin, parkinsonism-dementia complex (PDC)

Background: Amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia complex (PDC) have been highly prevalent in the Chamorro population of Guam. The cause of these disorders is unknown, although environmental neurotoxins with a long latency are thought to be possible causative factors. A remarkable neuropathological hallmark of PDC and Guamanian ALS is the constant and widespread distribution of neurofibrillary tangles (NFTs), suggesting these disorders have a common etiology or pathogenetic process. Numerous patients have been described with clinical features of both disorders. Studies of PDC patients have reported a high incidence of upper and lower motor neuron involvements. These findings support the view that Guamanian ALS and PDC constitute a single disease entity with a spectrum of clinical expressions. Several ALS studies of skin in patients with sporadic ALS have demonstrated morphological and biochemical abnormalities. However, there has been no study of skin in Guamanian ALS or PDC.

Objectives: We performed an ultrastructural study on skin biopsies in patients with Guamanian neurodegenerative diseases such as PDC and ALS.

Methods: Our subjects were 11 Guamanian patients with neurodegenerative disease, 11 clinically uninvolved Chamorro control subjects, 10 Japanese patients with sporadic ALS and 11 Japanese control patients with other neurologic or muscular diseases. Punch biopsy specimens of skin overlying the left biceps were taken. Electron micrographs were taken at 0.5 to 1.0 mm below the epidermal-dermal junction.

Results: For convenience, we have dealt with the Guamanian ALS and PDC cases as a single disorder. The diameter of collagen fibrils in sporadic ALS was significantly smaller ($P<0.001$) than in the Guamanian patients and controls and the Japanese controls. The diameter of collagen fibrils in Guamanian patients including one ALS patient was slightly smaller than that of Guamanian and Japanese controls, but this was not statistically significant ($P=0.60$). There was a significant negative correlation between the diameter of collagen fibrils and duration of illness in patients with sporadic ALS ($r=-0.81$, $P<0.01$), but there was no such correlation in Guamanian patients. In sporadic ALS patients, some areas showed that collagen bundles were widely separated by a large amount of amorphous material. The longer the duration of sporadic ALS, the more marked these findings. In the other three patient groups, all collagen fibrils were densely packed and parallel in collagen bundles without any increase in amorphous material.

Discussion and conclusions: It is suggested that Guamanian ALS and PDC differ from sporadic ALS. Our limited biopsy data could provide key elements to our understanding of the pathogenetic mechanisms of Guamanian ALS, PDC and of the difference between sporadic ALS, Guamanian ALS and PDC. Therefore, the skin studies reinforce the view of a different disease mechanism in Guamanian ALS and PDC as compared to sporadic ALS.

P67 RAB5 AND ITS ACTIVATOR ALS2 REGULATE AUTOPHAGOSOME MATURATION AND AUTOPHAGOLYSOSOME-MEDIATED PROTEIN DEGRADATION

OTOMO A, KUNITA R, IKEDA J-E, HADANO S

Department of Molecular Life Sciences, Tokai University School of Medicine, Isehara, Kanagawa, Japan

E-mail address for correspondence: asako@tokai-u.jp

Keywords: ALS2, Rab5, autophagosome

Background: Rab5 is a small GTPase which acts as a key regulator of several different modes of endocytosis and endosome trafficking. ALS2, the causative gene product for juvenile recessive motor neuron diseases, regulates macropinocytosis following endosome fusion. Recently, we reported that loss of ALS2 in $SOD1^{H46R}$ mice resulted in an earlier death and accelerated accumulation of abnormal autophagosomes in the spinal axons, indicating that malfunction in autophagolysosome-mediated protein degradation underlies *ALS2*-linked neurodegeneration. Since ALS2 functions as an activator for Rab5, ALS2-mediated Rab5 activation might be crucial for autophagosome maturation and autophagolysosome-mediated bulk protein degradation. However, little is known about the mechanism of autophagosome maturation, despite the protein machinery involving in the synthesis of autophagosomes being well described. Furthermore, it remains elusive as to whether Rab5 regulates autophagosome maturation.

Objectives: To understand the molecular pathogenesis associated with ALS2-deficiency, we investigated the molecular linkages of Rab5 and/or its activators to autophagosome maturation and autophagolysosome-mediated protein degradation.

Methods: We performed the siRNA-mediated gene silencing in HeLa and NSC-34 cells. Rab5A, Rab5B, Rab5C, ALS2, or RIN1 were individually, or simultaneously knocked down by the transfection with respective siRNAs. Knock-down efficiency of the target genes was evaluated by either real-time PCR or Western blot analysis. Expression levels of autophagosome markers such as LC-3 and p62/SQSTM1 were examined by Western blot analysis. Subcellular localization of LC3-positive autophagosomes was analyzed by immunocytochemistry.

Results: Western blot analysis demonstrated that siRNA-mediated simultaneous depletion of Rab5A, Rab5B and

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Rab5C (Rab5s), but not a single Rab5 protein, resulted in a clear elevation of lipidated form of LC-3 (LC3-II) and p62/SQSTM1 in HeLa cells. Further, depletion of Rab5s changed the localization of LC-3-positive autophagosomes from cell periphery to juxta-nuclear region, and caused the abnormal accumulation of autophagosomes. As expected, ALS2 depletion also led to the accumulation of LC-3-positive autophagosomes in the juxta-nuclear region. By contrast, transfection of control siRNA or siRNA for *RIN1*, another activator for Rab5, did not affect the subcellular localization and expression levels of LC3-II and p62/SQSTM1 in HeLa cells.

Discussion and conclusions: Our results indicate that Rab5s appear to function in autophagosome maturation and degradation, besides in the early endocytic pathway. Thus, attenuation of the Rab5 activity may impinge on autophagolysosome-mediated bulk degradation of cytoplasmic proteins and organelles. Interestingly, depletion of ALS2 but not RIN1 results in the autophagosome accumulation, suggesting that the Rab5 activation on a certain subtype of endosomes and/or autophagosomes by its regulator specifically functioning in autophagic pathway, such as ALS2, is required for the proper maturation and degradation of autophagosomes in cells. Further studies will provide insights not only into the mechanism of autophagosome maturation but also into the pathogenesis underlying *ALS2*-linked motor neuron diseases.

P68 DEVELOPMENT OF A NATIONAL ALS IPS CELL BANK FOR MOTOR NEURONS AND ASTROGLIA

ROTHSTEIN J[1], MARAGAKIS N[1], HENDERSON C[3], EGGAN K[2], MANIATIS T[2]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Harvard University, Boston, MA, United States,* [3]*Columbia Unviersity, New York, NY, United States*

E-mail address for correspondence: jrothstein@jhmi.edu

Keywords: iPS, astroglia, motor neurons

Background: To gain an understanding of the mechanisms underlying ALS requires access to relevant cell classes on the same genetic background. Living human motor neurons and astroglia have not been available for study. Mouse models although useful, employ highly over expressed human mutations in a non-human genetic background. iPS cells derived from human patients provide a potential but still largely unexplored approach to these shortcomings. As a first step toward the generalization of their use in the field, we are generating and validating new tools for the study of familial ALS. This will provide a strong basis for mechanistic and screening studies and for future extension of the approach to iPS cells from sporadic ALS patients.

Objective: We are generating 25 unique iPS lines from fALS skin biopsies, including various known ALS mutations: SOD1, TDP-43, FUS. Cell lines will be differentiated into motor neurons and astroglia, with a uniform and constant validation protocol. Finally all validated lines will undergo extensive genetic analyses including RNA seq. Final lines will be available to academic and commercial entities for pathogenesis research and drug screening.

Methods: A co-ordinated team approach is used to collect fALS skin biopsies from several academic sites. The fibroblasts generated from the biopsies are sent to iPierian for uniform generation of iPS cells with pMXS-VSVg retroviruses expressing KLF4, SOX2, OCT4 and cMYC. All final lines are charaterized initially for pluripotency such as expression of 20+ pluripotency genes (NANOG, SOX2, OCT4, etc), ability to form all three germ layers, and normal karyotyping. Final lines are submitted to the astrocyte core lab – for generation of astroglia (eg expression of mature astroglial markers, functional glutamate transport, invivo astroglial differentiation), and to the motor neuron labs – for generation and characterization of motor neuron phenotype. Finally each validated line undergoes extensive RNA profiling employing Solexa deep sequencing (RNA-Seq).

Discussion and conclusion: This national ALS iPS consortium is generating a comprehensive collection of human iPS derived motor neuron neurons and astroglia – all efficiently created under identical production methods. The ability to actually have human cell lines – representing the natural disease in the most relevant cell types – motor neurons and astrocytes – will provide unprecedented tools to 1) study cell-cell interactions responsible for disease pathophysiology and 2) provide critical tools for drug discovery and genetic pathway analysis. Eventually these ALS cell lines will also be useful to compare common and uncommon pathways between ALS and other neurodegenerative iPS models.

P69 MULTIPROTEIN BIOMARKERS OF AMYOTROPHIC LATERAL SCLEROSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS

BONETTO V[1,2], NARDO G[1,2], POZZI S[1,2], PIGNATARO M[1,2], LAURANZANO E[1,2], SPANO G[1,2], MONTEFORTE M[2], TORRI V[2], LUNETTA C[3], CORBO M[3], MORA G[4], BENDOTTI C[2]

[1]*Dulbecco Telethon Institute, Milano, Italy,* [2]*Mario Negri Institute for Pharmacological Research, Milano, Italy,* [3]*NEuroMuscular Omnicentre (NEMO), Niguarda Ca' Granda Hospital, Milano, Italy,* [4]*IRCCS Fondazione Salvatore Maugeri, Milano, Italy*

E-mail address for correspondence: valentina.bonetto@marionegri.it

Keywords: protein biomarkers, diagnosis/prognosis, PBMC

Background: For amyotrophic lateral sclerosis (ALS) there is still no diagnostic/prognostic test. The diagnosis is mostly based on clinical assessment with a history of progression of symptoms and is thus made with a delay of about a year from symptom onset, quite likely beyond the therapeutic window of a disease-modifying drug. Moreover, the clinical course varies widely. No ALS biomarkers are currently in clinical use but they would be valuable to support early diagnosis, monitor disease progression, and assess the efficacy of any new treatment.

Objectives: To identify a panel of reliable protein biomarkers of ALS in easily accessible peripheral blood mononuclear cells (PBMC) that exhibit traits of the disease in patients and animal models (1).

Methods: We used a proteome-based approach to examine PBMC of sporadic ALS (sALS) patients, non-ALS neurological patients and healthy individuals. In the analysis sALS patients were divided into two groups based on the levels of disease severity: low, with a functional rating scale score >24 and, high, with a score ≤ 24. The initial screening was done with pooled samples, then a panel of selected proteins was validated by dot blot on single samples of sALS patients ($n=60$) and healthy ($n=30$) and non-ALS neurological ($n=23$) controls, and on samples of PBMC and spinal cord of the G93A SOD1 transgenic rat model of ALS at different stages of the disease.

Results: We identified multiprotein biomarkers that are closely associated with ALS and can distinguish, with high discriminatory power, between two levels of disease

severity (90%), ALS patients from healthy controls (98%), and from patients with neurological disorders that may resemble ALS (91%). Some of them showed similar changes in PBMC of the rat model already at a pre-symptomatic stage of the disease and had similar behavior in the spinal cord.

Discussion and conclusions: Our multiprotein biomarkers are easily measurable in large-scale immunoassays and thus suitable to develop a helpful test in clinical practice. Some of the proteins identified in PBMC of sALS patients were detected also in the rat model of ALS and were previously found as hallmarks of disease in the central nervous system of ALS patients. This overlap endorses the use of PBMC for *in vitro* studies and reinforces the theory that ALS is a multicellular/multi-systemic disease. Finally, this is the first study that provides a highly feasible strategy to identify and validate multiprotein translational biomarkers in PBMC, potentially applicable to several neurological diseases, both for diagnosis and treatment.

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P70 SOD1 mRNA EXPRESSION INCREASE AS A BIOMARKER IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

GAGLIARDI S[1], COVA E[1], DAVIN A[1], GUARESCHI S[1], LAFORENZA U[4], CASHMAN JR[3], CERONI M[2,1], CEREDA C[1]

[1]*Laboratory of Experimental Neurobiology, IRCCS, Neurological Institute "C. Mondino", Pavia, Italy,* [2]*Division of General Neurology IRCCS, Neurological Institute "C. Mondino", Pavia, Italy,* [3]*Human BioMolecular Research Institute, San Diego, CA, United States,* [4]*Department of Physiology, Section of Human Physiology, University of Pavia, Pavia, Italy*

E-mail address for correspondence: cristina.cereda@mondino.it

Keywords: SOD1, mRNA

Background: Amyotrophic lateral sclerosis (ALS) is diagnosed on the basis of progressive symptoms in both the upper and lower motor neurons. The mutated Cu,Zn-superoxide dismutase gene (SOD1) is recognized as a pathological cause of 20% of the familial form of ALS. A mutation in the SOD1 gene can be considered as a genetic marker of ALS; no other specific biomarkers, as protein or gene expression, are available for ALS. Our previous results showed that lymphocytes from sporadic ALS patients possessed decreased SOD1 protein expression (1).

Objectives: mRNA SOD1 expression was examined in lymphocytes from SALS patients and controls to identify a possible SALS biomarker and to investigate whether protein expression decreasing was a consequence of decreased gene transcription (1). mRNA expression was evaluated in nervous tissues, in fibroblasts and in lymphocytes from SALS patients and in lymphocytes from Alzheimer's disease (AD) patients.

Methods: We examined *SOD1* gene expression by Taq-Man Real-Time qPCR in lymphocytes, brain subregions affected (cervical spinal cord, brain stem) and unaffected (cerebellum and non-motor cerebral cortex) by the disease and fibroblast cell lines from SALS patients and controls. Also lymphocytes from AD patients were analysed. SOD1 protein levels in lymphocytes, spinal cord and brain stem tissues from SALS patients and controls were measured by Western blot. Spinal cord specimens from SALS patients and controls were used for immunohistochemical analysis to evaluate SOD1 protein expression.

Results: We described a high level of SOD1 transcript in spinal cord, brain stem and lymphocytes of SALS patients ($P<0.001$) with respect to controls and AD patients. No differences in SOD1 mRNA levels were detected in unaffected ALS tissues. No correlation was found with the site of disease onset, disease duration or disease severity. Protein expression studies showed a similar or lower amount of SOD1 in affected brain areas and lymphocytes, respectively.

Conclusions: The observation of elevated mRNA SOD1 expression in specific nervous tissues typically affected by the disease (brain stem and spinal cord) shows that SOD1 upregulation is a pathological phenomenon and that lymphocytes have parallel motor neuron behaviour. The finding of normal levels of SOD1 mRNA in lymphocytes of AD patients indicates the specificity. A possible explanation for the discrepancy between mRNA and protein levels could be that SOD1 would change its structure and the misfolded protein could precipitate in the insoluble fraction as proteinaceous aggregates during protein extraction process (2).This hypothesis is in agreement with histochemical analysis showing higher SOD1 protein expression in SALS brain affected by disease. These findings provide new insight and understanding of the pathologic causes of SALS and allow a possible explanation for the molecular involvement of wild-type SOD1.

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P71 ALTERATION IN AMINO ACIDS IN MOTOR NEURONS OF THE SPINAL CORD IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

ISHIKAWA H, NOMURA M, YASUI K, MIKAMI H, WATANABE T, YAMANO T, SUZUKI M, ONO S

Teikyo University Chiba Medical Center, Ichihara/Chiba, Japan

E-mail address for correspondence: ono@med.teikyo-u.ac.jp

Keywords: amino acids, spinal cord

Background: Sporadic amyotrophic lateral sclerosis (SALS) is characterized pathologically by loss of motor neurons from the anterior horn of the spinal cord and a variable degree of corticospinal tract degeneration. However, the gracile and cuneate tracts of the posterior funiculus are well preserved in the spinal cord. Measurement of amino acids in precisely dissected regions of autopsied human spinal cord can provide useful information as to the biochemical basis of neurological disorders. Surprisingly little attention has been paid to possible abnormalities of amino acids in the spinal cords of SALS patients. At present, little is known concerning the changes in amino acid composition in different regions of the spinal cord in SALS.

Objectives: We describe here the results of quantitative amino acid analyses in precisely dissected regions of autopsied spinal cord from patients with SALS and compare the results with those in control subjects.

Methods: Our subjects were seven patients with SALS, seven patients with other neurologic diseases (control group A), and seven patients without neurologic diseases (control group B). At autopsy, blocks of cervical enlargement of the spinal cord were obtained from all the SALS patients and control groups A and B, and were immediately stored at -80°C. Continuous transverse sections of all blocks in each patient were separated by razor edge into the posterior funiculus, the lateral corticospinal tract, and the anterior horn. An aliquot of the hydrolysates was analyzed for its amino acid composition on

a Varian 5500 liquid chromatograph configured as an amino acid analyzer.

Results: The levels of collagen-associated amino acids, hydroxyproline, proline, glycine, and hydroxylysine, were markedly lower in the lateral corticospinal tract ($P<0.001$, $P<0.02$, $P<0.02$, and $P<0.02$) and the anterior horn ($P<0.01$, $P<0.02$, $P<0.01$, and $P<0.02$) in SALS patients than in control groups A and B. The contents of the acidic amino acids glutamate and aspartate were significantly decreased in the lateral corticospinal tract ($P<0.02$ and $P<0.05$) and the anterior horn ($P<0.02$ and $P<0.05$) of SALS patients as compared with those of control groups A and B.

Discussion and conclusions: Our study indicates abnormalities of collagen-associated amino acids and excitatory amino acids in the spinal cord in SALS, and this alteration exhibits a marked regional specificity, underlying the involvement of motor neurons of the spinal cord in this disorder. So far there have been no reports indicating changes in these amino acids in precisely defined areas of the spinal cord in SALS. Whether the decreased level of these amino acids is merely a secondary phenomenon, or has some bearing on the pathogenesis of SALS, remains a subject for further study.

P72 SURVIVAL MOTOR NEURON PROTEIN DEFICITS OCCUR IN ALS

TURNER B[1], TALBOT K[2], ANSORGE O[2], ATKIN J[1], HORNE M[1]

[1]*Florey Neuroscience Institutes, Melbourne, VIC, Australia,* [2]*University of Oxford, Oxford, United Kingdom*

E-mail address for correspondence: bradley.turner@florey.edu.au

Keywords: spinal muscular atrophy, survival motor neuron, sporadic ALS

Background: ALS and SMA selectively target anterior horn cells of the spinal cord, suggesting common factors that confer neuronal vulnerability in these disorders. While the genetics of ALS is complex, SMA results from reduced dosage of survival motor neuron (SMN) protein encoded by *SMN* genes. Genetic association studies also provide compelling evidence that abnormal *SMN* genotypes producing low levels of SMN protein significantly increase risk or severity of sporadic ALS. We recently demonstrated that SMN protein was depleted early in the disease course of cellular and mouse models of familial ALS and genetic ablation of *SMN* worsens progression in mutant SOD1 mice (1). These studies collectively raise the important question of whether SMN protein deficiency occurs in sporadic ALS patients.

Objectives: To investigate the expression level and cellular localisation of SMN and its binding partners in spinal cords of sporadic ALS patients.

Methods: Post-mortem lumbar spinal cord tissues were obtained from familial ALS cases (n=5), sporadic ALS patients (n=15) and non-neurological disease controls (n=5). Tissues were analysed by immunoblotting of protein extracts for SMN complex components and immunohistochemistry for SMN-positive nuclear gems in motor neurons.

Results: SMN and Gemin protein levels were severely depleted in spinal cord extracts of sporadic and familial ALS cases. SMN depletion correlated with disease progression, but not age of onset. SMN-immunoreactive gem counts were reduced accordingly in anterior horn cells of ALS patients.

Discussion and conclusions: These results establish that SMN protein loss occurs in motor neurons of sporadic ALS and correlates with clinical severity, consistent with genetic association data and observations from familial ALS models. Since SMN is functionally related to TDP-43 and FUS/TLS, these results emphasise the role of nuclear depletion of these RNA binding proteins as a potential common and upstream mechanism in ALS.

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P73 IMMUNOREACTIVITY OF SMAD UBIQUITINATION REGULATORY FACTOR-2 IN SPORADIC AND FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS AND ITS MOUSE MODEL

NAKAMURA M[1], KANEKO S[1], ITO H[2], ASAYAMA S[1], NISHII M[1], FUJITA K[1], KUSAKA H[1]

[1]*Department of Neurology, Kansai Medical University, Osaka, Japan,* [2]*Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan*

E-mail address for correspondence: nakamuma@takii.kmu.ac.jp

Keywords: Smad ubiquitination regulatory factor-2, phosphorylated Smad2/3, Tranforming growth factor-beta

Background: Smad ubiquitination regulatory factor-2 (Smurf2) is an E3 ligase belonging to the HECT-domain ubiquitin ligase family. Smurf2 interacts with Smad proteins and promotes their ubiquitin-dependent degradation, thereby controlling the cellular levels of these signaling mediators. Recently we reported that phosphorylated Smad2/3 (pSmad2/3), the central mediators of transforming growth factor (TGF)-beta signalling, abnormally accumulates in TAR DNA binding protein-43 (TDP-43) positive intracytoplasmic inclusions in sporadic amyotrophic lateral sclerosis (ALS) patients.

Objectives: As Smurf2 is an E3 ligase of phosphorylated Smad2, we aimed to determine the intracellular localization of Smurf2 in ALS.

Methods: Lumbar spinal cord sections from 8 sporadic ALS (SALS), 1 familial ALS (I113T:FALS), and 3 controls without neurological disorder were analyzed immunohistochemically. Lumbar spinal cord sections from G93A SOD1 transgenic (G93A Tg) female mice (mouse model of ALS) at 15-20 weeks of age and age-matched female wild-type littermates were also investigated immunohistochemically. The following primary antibodies were used: polyclonal antibodies against Smurf2 and pSmad2/3, and monoclonal antibodies (anti-phosphoserine 409/410) against phosphorylated TDP-43 (pTDP-43).

Results: Smurf2 immunoreactivity was hardly demonstrable in the cytoplasm of the control neurons. In all SALS patients, H&E staining revealed round hyaline inclusions (RHIs) and Bunina bodies. The RHIs and skein-like inclusions (SLIs) were immunopositive for Smurf2, but the Bunina bodies were devoid of labelling for it. A double immunofluorecence study for pTDP43 and Smurf2 revealed the co-localization of both within the RHIs and SLIs. In the FALS patient, neurofilamentous conglomerate inclusions (NFCIs) were present in the cytoplasm of the anterior horn cells, which were immunonegative for not only pSmad2/3 but also for Smurf2. In sections from control mice, Smurf2 immunoreactivity was observed in the cytoplasm of anterior horn cells. In the G93A Tg mice, the Lewy body-like hyaline inclusions (LBHIs) were

present in these cells but not labelled with the Smurf2 antibody.

Conclusions: This is the first demonstration of the presence of Smurf2 immunoreactivity in the pSmad2/3- and pTDP-43-positive inclusions in ALS. Our present results imply that the pSmad2/3-Smurf2 complex may play an important role in the pathomechanism underlying ALS.

P74 HYPERACTIVITY OF THE MITOCHONDRIAL RESPIRATORY CHAIN IN FIBROBLASTS OF PATIENTS WITH ALS

CAMPOS Y[1], MARTÍN R[1], ESTEBAN J[3], MORA J[2], MENDAÑA L[1], MERINO V[1], FERNÁNDEZ E[1], GARCÍA A[2], ALVAREZ JL[1]

[1]*Instituto de Salud Carlos III, Majadahonda/Madrid, Spain,* [2]*Hospital Carlos III, Madrid, Spain,* [3]*Hospital 12 de Octubre, Madrid, Spain*

E-mail address for correspondence: ycamposg@yahoo.es

Keywords: mitochondrial respiratory chain, glucose, hypermetabolism

Background: Amyotrophic lateral sclerosis (ALS) is a degenerative disorder characterized by a selective loss of upper motor neurons in the motor cortex and of lower motor neurons in the spinal cord and brainstem, culminating in respiratory insufficiency and death after 3-5 years. Despite extensive research, the cause of the disease is unknown in the majority of the cases, and the mechanisms of motor neuron injury are complex and are incompletely understood.
A proportion of patients with ALS exhibit a generalized hypermetabolism, characterized in the transgenic mice with mutations in the superoxide dismutase gene 1 (mSOD1 mice) by an increased muscular expenditure of glucose. On the other hand, a significant percentage of patients show glucose intolerance and mSOD1 mice develop a low cerebral consumption of glucose prior to symptom and pathology onset.

Objectives: The aim of this work is to study mitochondrial function in fibroblasts of patients with ALS when they grow in low or high glucose medium, to determine whether it varies with the availability of the substrate.

Methods: Skin fibroblast cultures were established from age-matched controls and patients (n=10 and n=6, respectively) after their informed consent. Respiratory chain enzyme activities, mitochondrial membrane potential, cell viability and duplication time were determined in cells growing in a medium with low glucose (1 g/L) or high glucose concentration (4.5 g/L).

Results: No differences were observed in any studied parameter in fibroblasts from controls, when they grew in a medium with different glucose levels. We found that fibroblasts of patients growing in low glucose medium had a significant increase in the activity of mitochondrial respiratory chain complexes I and IV when compared with control cells. No differences were detected in patients' cells cultivated in a rich glucose medium. However, the latter showed a significant drop in the membrane potential, cell viability and growth rate when compared to cells growing in low glucose concentration.

Conclusions: Fibroblasts from patients with ALS present an abnormal mitochondrial function when grown in different glucose concentrations. Considering that low glucose medium is the physiologic concentration in human blood (100 mg/100 mL) we hypothesise that fibroblasts of patients with ALS have an overactive mitochondrial respiratory chain in order to maintain membrane potential and the cell viability. This hyperactivity may be repressed by the presence of high glucose levels. Future studies will be necessary to explain this phenomenon.

P75 APOPTOSIS AND LOSS OF MITOCONDRIAL TRANSMEMBRANE POTENTIAL EVALUATED BEFORE AND AFTER INDUCTION OF OXIDATIVE STRESS IN PERIPHERAL BLOOD LYMPHOCYTES FROM SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

FROSALI S[1], GAGLIARDI A[1], LANDI C[1], BONGIOANNI P[2,3], ROSSI B[2], BINI L[1], DI MAIO G[4], FEDERICO A[4], DI STEFANO A[1]

[1]*Department of Molecular Biology, University of Siena, Siena (SI), Italy,* [2]*Azienda Ospedaliero-Universitaria Pisana, Pisa (PI), Italy,* [3]*NeuroCare onlus, Pisa (PI), Italy,* [4]*Department of Neurological, Neurosurgical and Behavioural Sciences, University of Siena, Siena (PI), Italy*

E-mail address for correspondence: paolo.bongioanni@tin.it

Keywords: mitochondrial transmembrane potential (MTP), apoptosis, lymphocytes

Background: Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease. Similar to other neurodegenerative disorders with incompletely defined etiology, such as Alzheimer's disease and Parkinson's disease, ALS appears to be a syndrome originating from diverse pathogenic processes. Only 10% of ALS cases seem to be associated with a familial course. Interestingly 20% of all ALS cases are caused by mutations of the copper-zinc superoxide dismutase (SOD1) gene. Multiple mechanisms have been implicated to explain motor neuron injury, including increased reactive oxygen species, altered mitochondrial function, glutamate excitotoxicity, altered calcium homeostasis, altered protein aggregation and proteosomal function, neuroinflammation and apoptosis. However, it is not clear which is the primary event or what the temporal relations are among these pathways. Recent evidence is building that actions on or originating in the mitochondria may be an important part of the disease. Mitochondrial dysfunction may cause motor neuron death by increasing generation of reactive oxygen species and by initiating the intrinsic apoptotic pathway.

Objectives: The present study was aimed to directly assess the spontaneous apoptosis and the susceptibility to undergo apoptosis of fresh human peripheral blood lymphocytes from 20 ALS patients compared to 20 healthy individuals. Apoptosis was induced by 2-deoxy-D-ribose (dRib), an agent which induces apoptosis in quiescent lymphocytes by interfering with cell redox status. The mitochondrial transmembrane potential (MTP) before and during dRib induced apoptosis was also investigated.

Methods: Isolated lymphocytes were cultured for 48 h and the time course (1 h, 24 h and 48 h) of both apoptosis and MTP modifications was monitored before and after dRib treatment by means of flow cytometry.

Results: Sporadic ALS lymphocytes showed increased levels of both spontaneous apoptosis and spontaneous MTP loss compared to normal lymphocytes. Interestingly, lymphocytes from patients with ALS showed a decreased susceptibility to apoptosis and a decreased tendency to lose MTP after dRib treatment. We observed a direct correlation between the severity of disease and minor propensity of lymphocytes to lose MTP during dRib -induced apoptosis in comparison to normal lymphocytes.

Discussion and conclusion: Our data reveal that lymphocytes from ALS exhibit: 1) increased level of spontaneous apoptosis and mitochondrial dysfunction, mirroring what has been found in motor neurons; 2) decreased susceptibility to oxidative stress induced by dRib that appears correlated to severity of disease. This could be likely due to a spontaneous adaptation of lymphocytes to oxidative stress, which has been already reported in ALS patients.

P76 THE BH3-ONLY PROTEIN BIM: POSSIBLE LINK BETWEEN ER STRESS AND APOPTOSIS IN CELLULAR MODEL OF ALS

SOO KY[1,2], FARG M[1], WALKER A[1,3], HORNE M[3,4], NAGLEY P[2], ATKIN J[1,3]

[1]*Department of Biochemistry, La Trobe University, Bundoora, Victoria, Australia,* [2]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia,* [3]*Brain Injury & Repair Group, Howard Florey Institute, University of Melbourne, Parkville, Victoria, Australia,* [4]*Centre for Neurosciences University of Melbourne, Parkville, Victoria, Australia*

E-mail address for correspondence: k.soo@latrobe.edu.au

Keywords: SOD1, mitochondrial apoptosis, ER stress

Background: We previously showed that mitochondrial apoptosis was correlated specifically with cells bearing mutant Cu,Zn-superoxide dismutase 1 (SOD1) inclusions in ALS. However, dispersed SOD1 proteins, either wildtype (WT) or mutant, were found to partially protect cells against apoptosis, and this protection is upstream of mitochondria engagement in apoptosis. ER stress was also found to be linked to neurotoxicity associated with mutant SOD1 inclusions.

Objective: We demonstrate whether the BH3-only protein, Bim, is a direct link between ER stress and mitochondrial apoptosis in ALS.

Methods: Depletion of Bim by siRNA technology was used in Neuro2a cells, a mouse neuroblastoma cell line, expressing either WT or mutant SOD1 at 72 h post transfection. Immunostaining and confocal microscopy were performed to investigate both mitochondrial apoptotic and ER stress markers. Cells expressing WT SOD1 were treated with apoptotic inducers for a further 24 h and changes of nuclear morphology were determined. For kinetic studies, NSC-34 cells expressing WT or mutant SOD1 were examined from 10 to 24 h post transfection and before inclusion formation. Pro-apoptotic markers, such as translocation of CHOP to nucleus and recruitment of Bax to mitochondria, were investigated by immunostaining and confocal microscopy.

Results: In Neuro2a cells, Bim knockdown by siRNA significantly reduced nuclear apoptotic features in cells bearing mutant SOD1 inclusions. After Bim knockdown, both Bax recruitment to mitochondria and cytochrome c redistribution were also decreased in such inclusion-bearing cells. However, CHOP translocation to nucleus, a marker of ER stress, was not reduced by Bim knockdown. Significantly, the neuroprotection afforded by dispersed WT SOD1 was substantially enhanced by Bim-depletion, observed in Bim-depleted cells exposed to various apoptotic insults. In cells not subjected to Bim knockdown, kinetic studies indicated CHOP translocation to nucleus to occur prior to formation of mutant SOD1 inclusions. Interestingly, Bax recruitment to mitochondria (but not apoptotic nuclei) was also observed before formation of mutant SOD1 inclusions.

Discussion and conclusion: These data provide the evidence that Bim plays an important role in SOD1-linked ALS. Furthermore, the findings from this study also suggest that neurotoxicity in ALS is induced by a toxic structure derived from mutant SOD1 that activates stress responses in cells much earlier than the appearance of grossly aggregated SOD1 in inclusions.

P77 ELEVATION OF ANGIOPOIETIN RECEPTOR TIE-2 IN PLASMA FROM PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

ZHANG R[1], HONRADA R[1], MADISON C[2], CHAMPION S[2], KATZ J[2], FORSHEW D[2], MILLER RG[2], MCGRATH MS[1]

[1]*University of California, San Francisco, San Francisco, CA, United States,* [2]*California Pacific Medical Center, San Francisco, CA, United States*

E-mail address for correspondence: mmcgrath@php.ucsf.edu

Keywords: monocyte/macrophage activation, inflammation/activation markers, Tie-2

Background: Our previous studies demonstrated elevated blood levels of abnormally activated monocyte/macrophages in sALS. Elevated plasma Lipopolysaccharide (LPS) levels correlated with degree of monocyte/macrophage activation and levels of plasma monocyte/macrophage activation markers sCD14 and sCD163. These data confirmed the inflammatory nature of sALS. Recent studies have implicated angiopoietin receptor Tie-2 as well as vascular endothelial growth factor (VEGF) in the pathogenesis of inflammatory and autoimmune diseases. Although Tie-2, is mainly expressed by endothelium, it is also expressed in monocytes participating in the development of angiogenic and inflammatory disease processes. Angiogenic molecules known to control blood vessel growth and proliferation appear also to regulate neuronal process development. These data linking sALS as an inflammatory disease with inflammation associated markers of angiogenesis suggest that Tie-2 and its level may be involved in sALS pathogenesis.

Objectives: 1) To quantify levels of plasma Tie-2 in sALS patients as compared to control groups; 2) To determine if plasma levels of Tie-2 correlate with clinical stage of disease in sALS.

Methods: Tie-2 ELISA was performed to quantify plasma levels of Tie-2 in heparinized blood samples from 15 sALS, 10 Alzheimer's (AD), and 15 healthy controls (HC). Results from this immune study were evaluated in light of the severity of neurological impairment as determined by ALSFRS-R scores.

Results: Compared to HC (16.7 ± 2.7 ng/ml), significantly higher levels of plasma Tie-2 were identified in sALS (20.4 ± 5.2 ng/ml, $P<0.05$) and AD (22.2 ± 6.0 ng/ml, $P<0.05$). Plasma Tie-2 levels between two disease groups, sALS and AD, were similar. Similar elevated patterns were observed in our previous studies on monocyte/macrophage activation markers sCD14 and sCD163 in sALS and AD patient specimens. Plasma Tie-2 and levels of monocyte/macrophage activation markers in sALS, AD and HC were significantly correlated (sCD14: $r=0.5346$, $P=0.0004$; sCD163: $r=0.4544$, $P=0.0079$). There was a trend associating increased levels of plasma Tie-2 with ALS disease severity: patients with more marked impairment had the highest Tie-2 levels (ALSFRS-R score=25-36, n=9, $P<0.05$) compared with those with mild impairment (ALSFRS-R score=37-48, n=6); but no difference was found between HC and sALS patients with mild impairment.

Conclusions: This study, for the first time, reveals that plasma levels of Tie-2 are significantly elevated in patients

with sALS, and plasma Tie-2 levels might be associated with ALS disease severity. Increased levels of Tie-2 were significantly correlated with degree of monocyte/macrophage activation markers. These findings not only demonstrate a role for angiopoietin/Tie system in ALS pathogenesis but also suggest that a complex array of inflammation/activation-related markers might be able to be used to monitor progressive sALS disease and potential response to therapeutic intervention in patients with sALS.

P78 MODIFICATIONS AND AGGREGATION OF TRANSTHYRETIN IN ALS

COLLINS M, KOLARCIK C, BOWSER R

University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

E-mail address for correspondence: bowserrp@upmc.edu

Keywords: transthyretin, protein aggregation, cerebrospinal fluid

Background: Transthyretin is a homotetrameric protein found in the blood and cerebrospinal fluid (CSF) that regulates the transport of biological molecules, including thyroxine and vitamin A. Mutations in the TTR gene result in familial amyloidoses, whereby TTR protein is deposited extracellularly. This can induce peripheral neuropathy and autonomic dysfunction. These protein deposits contain TTR amyloid-beta structured fibrils. Post-translational modifications to TTR influence the protein's propensity to aggregate and have been observed in Alzheimer's disease. Our prior studies have identified a decrease in the native monomer form of transthyretin (TTR) in the cerebrospinal fluid (CSF) of ALS patients. In addition, we detected increased levels of specific post-translational modifications to TTR in the CSF of ALS patients. The current study was performed to further characterize TTR in the CSF and spinal cord tissue of ALS patients.

Objective: To characterize modifications and protein aggregation state of TTR in CSF and spinal cord tissue of ALS patients.

Methods: CSF was obtained from ALS and healthy control subjects. TTR was isolated from CSF by immunoprecipitation or column chromatography. For immunoprecipitation experiments, TTR was incubated with polyclonal TTR antibody (DAKO) and conjugated to paramagnetic protein A/G microbeads which were then loaded into an elution column. The labeled immune complex was eluted with SDS sample buffer. For column chromatography experiments, CSF was first run through a multiple affinity removal column (Agilent) to remove abundant CSF proteins. The depleted fractions containing TTR were then run through a size exclusion column to separate TTR species of various molecular weights. Fractions from the size exclusion column were saved, desalted, and analyzed by Western blot. Fresh frozen and fixed spinal cord tissue was obtained at time of autopsy from 12 ALS and 5 non-neurologic disease control subjects. We measured levels of TTR in the CSF by ELISA and mass spectrometry. We also performed immunohistochemistry for TTR in post-mortem spinal cord tissue sections. Immunoblotting was performed on 10-12% BIS/TRIS gels. Proteins were transferred from the gels to polyvinylidene fluoride membranes. Membranes were blocked in 2% nonfat milk for 1 hour, incubated in primary antibody overnight (TTR polyclonal; DAKO), incubated in secondary antibody (donkey anti-rabbit), and visualized by ECL.

Results: We verified our prior results indicating decreased levels of native TTR monomer in the CSF of ALS patients, with concurrent increased levels of TTR containing specific post-translational modifications. We observed increased levels of high molecular weight TTR in the CSF of ALS patients.

Conclusions: Within the CSF, TTR post-translational modifications are altered during ALS. These findings suggest that TTR aggregation occurs during ALS. The presence of extracellular TTR aggregates in ALS would be a novel pathologic finding in ALS, suggesting novel pathogenic mechanisms of disease.

P79 EXTRACELLULAR MATRIX AND CELL ADHESION ALTERATIONS IN ALS

COLLINS M, BOWSER R

University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

E-mail address for correspondence: bowserrp@upmc.edu

Keywords: extracellular matrix, mass spectrometry, neuropathology

Background: The extracellular matrix (ECM) forms a critical component of cellular communication, protection and adhesion with neurons and non-neuronal cells within the central nervous system. The ECM provides important structure to the synapse, provides guidance during neuronal migration and axonal outgrowth/regeneration, binds numerous growth factors and modulates their release and delivery to cell surface receptors. In addition, the ECM forms perineuronal nets that provide neuronal protection and regulate synaptic plasticity. Little work has been done to examine the ECM and perineuronal nets in the human spinal cord and how their disruption may affect neuronal survival. Therefore, we examined the ECM in the spinal cord of ALS and control subjects.

Objective: To characterize extracellular matrix proteins in the CSF and spinal cord tissue of ALS patients and control subjects.

Methods: CSF was obtained from 250 ALS, disease control and healthy control subjects. Liquid chromatography based mass spectrometry (LC-MS/MS) was performed to identify ECM proteins and extracellular matrix proteases in the CSF of ALS and control subjects. Fresh frozen and formalin fixed spinal cord tissues were obtained at the time of autopsy from 12 ALS and 5 non-neurologic disease control subjects. We measured levels of ECM proteins in frozen spinal cord tissue homogenates by immunoblot. Immunohistochemistry for ECM proteins was also performed in post-mortem spinal cord tissue sections.

Results: By LC-MS/MS, we identified altered levels of specific ECM proteins in the CSF of ALS patients, including tenascin-R, specific collagen isoforms, fibronectin, and matrix metalloproteinases (MMPs). We also observed altered distribution of ECM proteins in the spinal cord of ALS patients by immunohistochemistry. Perineuronal nets appeared disrupted around spinal cord motor neurons in ALS patients. We also detected altered subcellular distribution of cell adhesion proteins within motor neurons during ALS.

Conclusions: Our results indicate that the extracellular matrix within the central nervous system (CNS) is significantly altered during ALS. Disruption of the ECM may directly impact neuronal survival, synaptic integrity, neuronal plasticity and regeneration, and affect the vasculature within the CNS.

P80 THE EFFECT OF ALS IGG ON INTRACELLULAR CALCIUM IN CULTURED PYRAMIDAL NEURONS AND ASTROCYTES

MILOSEVIC M[1], STEVIC Z[2], ANDJUS P[1]

[1]*Institute for Physiology and Biochemistry, School of Biology,* [2]*Institute of Neurology, School of Medicine; University of Belgrade, Belgrade, Serbia*

E-mail address for correspondence: zsmnd.yu@sezampro.rs

Keywords: IgG, Ca2$^+$ homeostasis, excitotoxicity

Background: It was suggested that IgG of ALS patients cause excitotoxicity by acting on voltage-gated Ca^{2+} channels. We have previously shown that ALS IgGs increase the frequency of spontaneous glutamatergic synaptic activity in rat hippocampal neurons in culture (1) and of glycine-mediated IPSCs in hypoglossal motor neurons in rat brain-stem slices (2). It was also shown that ALS IgGs can modify neuronal Ca-transients (3).

The aim of the study: to measure the direct effect of the IgG fraction from sera of sporadic ALS patients on intracellular calcium in hippocampal pyramidal neurons and cortical astrocytes in culture.

Patients and methods: The IgG fraction was isolated from 6 sporadic ALS patients and 5 controls (pathologic and normal). IgGs were purified by affinity chromatography (protein A-sepharose) as described in (1). ALS IgGs dialized towards the experimental medium were applied with a hand micropipette to the measuring chamber by adjusting the applied volume to reach 0.1 mg/ml of final concentration. Pharmacological agents were added to the chamber by perfusion. Hippocampal neurons and cortical astrocytes were dissociated from PND 2-5 Wistar rats and cultured on glass coverslips. Ca-imaging was performed with the fluorescent dyes Fluo 3 and 4 AM.

Results: Most of the ALS IgG samples regularly induced an acute Ca^{2+} response with a fast transient often followed by a slow and prolonged phase lasting several minutes. Depending on the IgG sample the second phase could be transient or result in a stable increase in intracellular Ca^{2+}. In cases of recovery Ca^{2+}-rise could be induced again by KCl. The response in cultured cortical astrocyte was more of the transient nature. The transients were shown to be dependent in neurons on the state of lysosomes (by their disruption with 0.2 mM Gly-Phe-beta-naphtylamide), and in neurons as well as astrocytes on Ca^{2+}-stores (by the use of 1 μM thapsigargine), but also on Ca^{2+} influx (with Ca^{2+}-free external solution).

Conclusion: Although qualitative, these observations confirm the general excitotoxic effect of ALS IgGs on Ca^{2+} homeostasis in non-motor neurons as well as in non-neuronal cells. The efect is compelx involving intracellular Ca^{2+} stores as well as trans membrane Ca^{2+} influx.

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P81 NFL MICRORNA EXPRESSION PROFILE IN SPORADIC ALS

STRONG M[1,2], HE Z[1], CAMPOUS D[1]

[1]*Robarts Research Institute,* [2]*Department of Clinical Neurological Sciences; The University of Western Ontario, London, Ontario, Canada*

E-mail address for correspondence: mstrong@uwo.ca

Keywords: RNA, neurofilament

Background: A neuropathological hallmark of ALS is the formation of neurofilamentous aggregates due to alterations in the stoichiometry of the individual neurofilament (NF) subunit proteins that includes a selective suppression of the low molecular weight NF (NFL) steady state mRNA levels relative to those of the intermediate molecular weight (NFM) and high molecular weight (NFH) steady state mRNA levels. We have shown that degenerating ALS motor neurons (identified by increased TDP-43 expression) express elevated levels of P-bodies (RNA granules that target mRNA for degradation) and that these granules contain NFL mRNA. Recognizing that the trigger for P-body formation is the conversion from a stress granule (translationally silent RNA granules), we sought to determine whether the NFL miRNA expression profile differs between ALS and control spinal cord.

Objectives: To characterize the expression profile of miRNA that interact with NFL mRNA 3'UTR and determine whether this profile differs between ALS and control.

Methods: We used two different prediction algorithms (Target Scan, MiRanda) to develop a panel of highly conserved miRNAs that recognize the human NFL mRNA 3'UTR. This yielded 103 distinct miRNAs that would potentially interact with the NFL mRNA 3' UTR. Of these, 19 were highly conserved. We extracted miRNA from ventral lumbar spinal cord lysates of control (3) or *s*ALS (7) using the MirVana miRNA isolation kit and then examined miRNA copy number in the ventral lumbar spinal cord for each of the 103 predicted NFL mRNA interacting miRNAs. Copy levels were determined by quantitative real-time PCR (RT-qPCR; SYBR green), quantified using known standards, and expressed as high (10^7 - 10^8 copies), intermediate (10^4 – 10^6 copies), low copy number (10^1 – 10^3 copies) or absent. Differences between miRNA expression levels amongst cases were analyzed using Real-Time StatMiner version 3.0 (Integromics). Physical interaction between the miRNA of interest and NFL mRNA was confirmed by EMSA, and functional interaction using the luciferase assay.

Results: From among the 103 miRNAs predicted to interact with NFL mRNA, we observed only 4 differentially expressed between ALS and control. This included a reduction in copy number for miR 188-3p ($P<0.01$) and miR 92-2* ($P < 0.05$) and an increased copy number for miR23a* and miR23b* ($P \leq 0.01$). Both EMSA and luciferase assays are in progress, although for the latter, 2 of the 4 miRNAs induce an enhanced rate of degradation of NFl mRNA when co-expressed with the luciferase construct in HEK 293T cells.

Conclusions: These observations suggest that the suppressed levels of NFL mRNA observed in ALS spinal motor neurons is related to an alteration in miRNA expression favoring an enhanced rate of mRNA degradation mediated by P-bodies.

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P82 HETEROGENEITIES OF MUSCLE PATHOLOGY FROM ALS/MND PATIENTS

AL-SARRAJ S[1], CLEVELAND M[2], PRADAT P-F[3], CORSE A[4], ROTHSTEIN J[4], LEIGH N[1], BATES S[2], WURTHNER J[2], MEININGER V[3]

[1]*King's College Hospital, London, United Kingdom,* [2]*GSK Biopharm R&D, Stevenage, United Kingdom,* [3]*Hôpital de la Salpêtrière, Paris, France,* [4]*Johns Hopkins University, Baltimore, United States*

E-mail address for correspondence: jens.u.wurthner@gsk.com

Keywords: mitochondrial abnormalities, myofibrillary architecture, muscle pathology

Background: Amyotrophic lateral sclerosis (ALS) is a primary degeneration of upper and lower motor neurones. However, it is now recognised that the degeneration is more widespread throughout the CNS with spectrum of changes involving, in addition, the brainstem and cerebral cortex. Muscle biopsies from patients with ALS are known to show neurogenic changes with de-enervation and re-enervation processes. The extent of pathology depends on the degree of loss of anterior horn cells. There is no recent major review of myopathology of ALS patients.

Objectives: To comprehensively examine muscle pathology from a cohort of clinically confirmed ALS patients and to investigate various markers on inflammation, complement activation and deposition of abnormal proteins and compared them with an age matched control.

Methods: Thirty one muscle biopsies from clinically confirmed ALS patients alongside 20 normal controls were stained for comprehensive protocol of histochemical stains in addition to HLA-ABC, utrophin, neo-myosin, C5b-9, p62, TDP-43 and desmin.

Results: The neurogenic changes of various degree and extent were confirmed in 29/31 cases with another case of possible neurogenic changes. In one case there were no features of neurogenic changes. 3/31 cases showed necrosis of muscle fibres with inflammatory infiltration and HLA-ABC over-expression. A further 2 cases showed over-expression of HLA-ABC without evidence of inflammatory cell infiltration. In 4/31 cases there was significant deposition of complement (C5b-9) in the small endomysial capillaries. Mitochondrial abnormalities were seen in 3/31 cases more than those expected in normal ageing. Many biopsies show severe disruption of myofibrillary architecture and 13/31 cases show cytoplasmic bodies; some of these were faintly and focally stained with p62 and desmin but none were immunoreactive to TDP-43.

Discussion and conclusions: Although neurogenic changes are confirmed in the majority of ALS patients, there are additional pathologies including possible low-grade inflammation and mitochondrial abnormalities which may give an alternative insight into the management of ALS.

P83 AUTOPHAGY IN MOTOR NEURONS OF THE SPINAL CORD IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

SASAKI S

Tokyo Women's Medical University, Tokyo, Japan

E-mail address for correspondence: ssasaki@nij.twmu.ac.jp

Keywords: autophagy, autophagosome, autolysosome

Background: Autophagy is a cellular pathway in the bulk degradation and recycling of cytoplasmic constituents to maintain cellular homeostasis. Autophagy is induced in response to cellular stress, and may either serve as a cell survival mechanism or play a role in cell death. Dysregulation of autophagy is considered to play a role in neurodegeneration. Of autophagy in amyotrophic lateral sclerosis (ALS), very little is known, and in particular, the precise fine structural evidence of autophagy and involvement of autophagy in the pathomechanism of neurodegeneration of motor neurons remain unknown.

Objectives: To examine electron-microscopically if autophagic processes are observed in the cytoplasm of motor neurons of the spinal cord in patients with sporadic ALS, and if autophagy is involved in the pathomechanism of the neurodegeneration of motor neurons in this disorder.

Methods: Autopsy cases were examined in which postmortem investigations had been performed within 6 h of death to avoid delayed postmortem artifacts to the utmost. Electron-microscopically, 16 patients with sporadic ALS (aged 49-83 years; mean age 68.7 ± 8.5 years) and 15 age-matched control patients (aged 44-80 years; mean age 62.7 ± 11.2 years) were studied who died without having any known neurological disease. The lumbar spinal cord (L1-L5) was fixed in 2% glutaraldehyde with phosphate buffer (pH 7.40) at the autopsy. After conventional procedures, ultrathin sections, which were stained with uranyl acetate and lead citrate, were studied by electron microscope.

Results: In ALS, all patients showed autophagic processes in the cytoplasm of normal-appearing motor neurons and more frequently degenerated motor neurons with central chromatolytic change. In particular, the patients with a short clinical course and relatively well preserved anterior horn neurons showed autophagy more frequently. Autophagosomes surrounded by a unique double-membrane and autolysosomes isolated by a single membrane contained the sequestered cytoplasmic organelles such as mitochondria and ribosome-like structures. Autophagy was also found in frequent association with ALS-characteristic inclusions: Lewy body-like inclusions contained autophagosomes and autolysosomes inside and/or at the periphery; autophagosome or autolysosome engulfed tiny or relatively large skein-like inclusions; Bunina bodies contained autophagic structures. Moreover, other abnormal structures such as the honeycomb-like structure showed autophagy occasionally. In the controls, normal-looking motor neurons showed no autophagic processes in the cytoplasm.

Conclusions: These findings suggest that moderate up-regulation of autophagic activity at the early stage of motor neuron degeneration may play a protective role, whereas at the later stage, excessive autophagy beyond the ability of clearance of protein aggregates may contribute to neurodegeneration of motor neurons, eventually leading to autophagic cell death in sporadic ALS.

P84 INTRANUCLEAR AGGREGATES OF SUPEROXIDE DISMUTASE-1 IN GLIAL CELLS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

FORSBERG K, ANDERSEN P, MARKLUND S, BRÄNNSTRÖM T

Umeå University, Umeå, Sweden

E-mail address for correspondence: karin.forsberg@medbio.umu.se

Keywords: nucleus, glial cells, superoxide dismutase-1

Background: The most common cause of amyotrophic lateral sclerosis (ALS) is mutations in superoxide dismutase-1

(SOD1). There is accumulating evidence for involvement of non-neuronal cells in the pathogenesis of ALS. In murine SOD1 models, it has been shown that down-regulation of SOD1 expression in astrocytes and microglia slow progression after disease onset, whereas down-regulation in motoneurons delays onset of disease. The exact role of non-neuronal cells in the pathogenesis of ALS is unknown but all cells implicated in the transgenic models show signs of activation or alteration in the human disease.

Objective: Here we searched for signs of involvement of SOD1 in the pathogenesis of ALS with special focus on glial cells.

Methods: Spinal cord from nine ALS-patients carrying SOD1-mutations, from 51 patients with sporadic or familial ALS who lacked such mutations, and from 46 controls, 20 non-neurological diseases and 26 neurodegenerative diseases, were examined by immunohistochemistry. An in-house set of anti-peptide antibodies with very high specificity for misfolded SOD1 was used.

Results: Immunoreactivity for misfolded SOD1 was regularly detected in the nuclei of ventral horn astrocytes, oligodendrocytes, and microglia both in ALS patients carrying SOD1 mutations and in sporadic and familial patients lacking such mutations. Misfolded SOD1 were seen as numerous immunopositive intranuclear granular aggregates approximately measuring 0.5-2 μm. There was only negligible staining in control patients with neurodegenerative or non-neurological disease. Staining for misfolded SOD1 staining was also seen occasionally in nuclei of motoneurons of ALS patients.

Discussion and conclusion: The results suggest that misfolded SOD1 present in glial and motoneuron nuclei may be involved in the pathogenesis of ALS.

P85 SOD1 PROTEIN LEVELS IN THE CSF OF ALS PATIENTS

WINER L[1], CHUN S[1], JAFFA M[2], LACOMIS D[3], BOWSER R[3], CUDKOWICZ M[2], MILLER T[1]

[1]*Washington University, St. Louis, MO, United States,* [2]*Massachusetts General Hospital, Boston, MA, United States,* [3]*University of Pittsburgh, Pittsburg, PA, United States*

E-mail address for correspondence: millert@neuro.wustl.edu

Keywords: CSF, SOD1, antisense oligonucleotides

Objective: To determine levels of SOD1 in the CSF of ALS patients versus controls.

Background: Mutations in SOD1 cause a proportion of familial ALS and there is increasing evidence that SOD1 is involved in sporadic ALS. Treatments are being developed to decrease levels of SOD1 as a treatment for familial ALS. Understanding whether SOD1 levels are different in ALS patients versus controls will provide an important background for understanding the effect of medications that decrease SOD1 and for understanding whether SOD1 may be part of the pathogenic process in ALS.

Design and methods: In CSF from 100 patients with sporadic ALS, 42 healthy controls, and 50 neurological disease controls, we measured SOD1 protein levels by ELISA assay and total protein by colorimetric assay. CSF samples were provided by the Northeastern ALS Consortium Sample Repository. Neurological disease controls included Alzheimer's (11), peripheral neuropathy (15), multiple sclerosis (19), and upper motor neuron only (5). In a separate group of 7 patients, CSF was collected every 6 months for approximately 2 years. SOD1 protein levels were measured.

Results: SOD1 protein levels were increased (182 +/- 78 ng/ml) in sporadic ALS patients compared with healthy controls (135 +/- 54 ng/ml) or neurological disease controls (140 +/- 31 ng/ml), ($P<0.05$). Total protein levels were increased in ALS patients (72+/-20 mg/dL vs healthy controls (58 +/- 15 mg/dL), $P<0.05$, but not significantly changed relative to neurological disease controls (68+/-22 mg/ml), ($P>0.05$). Repeat measurements of SOD1 in the same patient at different time points showed less than 20% variation in all patients.

Conclusions: SOD1 protein levels are elevated in CSF from sporadic ALS patients compared with healthy controls and neurological disease controls, suggesting that SOD1 may be part of the pathological process in sporadic ALS. Total protein is also increased in CSF from ALS patients compared with healthy controls. These results will be considered in terms of ALS disease characteristics. In repeat samples at different time points, SOD1 protein levels appear relatively stable.

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P86 EVIDENCE OF NUCLEAR RELOCALIZATION OF WILD-TYPE SOD1 UNDER STRESSFUL CONDITIONS

GUARESCHI S[1], MILANI P[1], BIGI R[1], ALVISI E[2], BIANCHI M[1], CERONI M[1,2], CEREDA C[1], COVA E[1]

[1]*Laboratory of Experimental Neurobiology,* [2]*Division of General Neurology; IRCCS, Neurological Institute "C. Mondino", Pavia, Italy*

E-mail address for correspondence: cristina.cereda@mondino.it

Keywords: SOD1, nucleus, oxidative stress

Background: It has been suggested that wild-type SOD1 (WTSOD1) may acquire, following oxidative damage, aberrant and/or toxic properties of mutant SOD1 and be implicated in a fraction of sporadic ALS cases (SALS), which represent 90% of ALS patients. We have previously demonstrated that SALS patients' lymphocytes express decreased SOD1 protein albeit mRNA expression is significantly higher compared to control. These findings prompted us to investigate the discrepancy between the presence of low protein amount and high messenger expression by assaying the hypothesis of a different compartmentalization of SOD1 in patients.

Aim: In this work we assayed the presence of an altered distribution of WTSOD1 between nucleus and cytosol in SALS lymphocytes. Moreover SOD1 distribution was studied in a cell line under stressful conditions and compared with WTSOD1 in SALS lymphocytes.

Methods: Lymphocytes were isolated from SALS and control subjects by Ficoll gradient. SH-SY5Y (SH) cells were maintained in DMEM/F-12 medium and treated with 1 mM H_2O_2. Confocal analysis: cells were plated and fixed using 10% paraformaldehyde. Samples were incubated o/n with anti-SOD1 antibody and then with secondary antibody. Flow cytometry: increased free radicals in nucleus and cytoplasm and cell damage were evaluated by dihydro-rhodamine 123 and Apocons kit, respectively. Nuclear and cytosolic SOD1 protein expression was analyzed by Western blot (WB). Immunoprecipitation: after pre-clearing samples were incubated with an anti-SOD1 antibody. The antibody-antigen complexes were analyzed by WB.

Results: Flow cytometry experiments showed a significantly higher amount of free radicals (ROS) both in nucleus and cytosol of SALS lymphocytes compared to controls. A significantly larger fraction of SALS lymphocytes was apoptotic. Higher SOD1 protein expression was found in SALS nuclei than in control cells, as demonstrated by WB and confocal microscopy. Cytosolic SOD1 protein level was unchanged in SALS and controls. SH cells, upon treatment, showed an expression pattern similar to SALS. Immunoprecipitation experiments demonstrated that in SALS lymphocytes SOD1 is more oxidized than in control cells as detected in SH cells upon oxidative stress.

Discussion: In this work we proved an altered SOD1 intracellular distribution in SALS lymphocytes compared to control cells. The same pattern of expression was detected in a human neuroblastoma cell line after H_2O_2 treatment, suggesting that this redistribution could be a physiological response to increased oxidative stress. Higher apoptosis rate and ROS amount in nuclear and cytosolic compartments of patients sustain this hypothesis and indicate that WTSOD1 role in the nucleus need to be investigated. Although the observation of high level of oxidised SOD1 in SALS does not solve the question if it is the cause or the consequence of increased oxidative stress, it proves higher WTSOD1 oxidation in SALS patients.

P87 EVIDENCE OF ABNORMAL OXIDATION OF SOD1 PROTEIN IN LYMPHOBLASTS OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS PATIENTS AND IN AN ALS CELLULAR MODEL BY SELDI-TOF-MS TECHNOLOGY

GHIROLDI A[1,2], COVA E[1], BARBARINI N[3], LISTA S[1], GUARESCHI S[1], CERONI M[1,4], MAGNI P[3], CEREDA C[1]

[1]*Laboratory of Experimental Neurobiology, IRCCS, National Neurological Institute "C. Mondino", Pavia, Italy,* [2]*Department of Neurosciences, University of Pavia, Pavia, Italy,* [3]*Department of Computer Science and Systems, University of Pavia, Pavia, Italy,* [4]*Division of General Neurology IRCCS, Neurological Institute "C. Mondino", Pavia, Italy*

E-mail address for correspondence: cristina.cereda@mondino.it

Keywords: SELDI-TOF-MS, SOD1, oxidation

Background: Recent studies suggest that superoxide dismutase (SOD1) may represent a major target of oxidative damage in neurodegenerative diseases. It has also been demonstrated that WT-SOD1 may acquire toxic properties of SOD1 mutant forms through oxidative damage (1). These modifications could alter protein stability, enzyme activity and redox state (2). We used a proteomic approach to study SOD1 post-translational modifications (PTM): SELDI-TOF-MS is a proteomic technique that combines chromatography and mass spectrometry, it is used for biomarker discovery and to study PTMs (3).
In this study we highlighted the presence of increased SOD1 oxidation in sALS patients' lymphoblasts with SELDI-TOF-MS approach and advanced bioinformatic analysis of peak decomposition.

Methods: *Oxidative stress:* To identify the SOD1 peak corresponding to oxidation, purified SOD1 from human erythrocyte (hSOD1) and SH-SY5Y (SH) cells were incubated for 1 hour with hydrogen peroxide 1 mM. *SELDI-TOF-MS analysis:* 5 micrograms of protein extracted with RIPA buffer from lymphoblasts of sALS patients, healthy controls and human neuroblastoma cell line SH were loaded onto IMAC30 array functionalized with Cu^{2+} and analyzed using SELDI-TOF-MS. hSOD1 was employed as standard to properly recognize SOD1 forms. A bioinformatic innovative procedure was applied to decompose the SOD1 broad low resolution peak, in order to determine overlapping isotopic distributions (peaks) and to identify SOD1 PTMs.

Results: Analysis of hSOD1 peaks showed the presence of seven different peaks representing seven protein PTMs. Analysis of oxidized hSOD1 presented the same pattern of decomposition, but showed an increase in the peak at 15.885 Da that differed 30 Da compared to the main SOD1 peak at 15.855 Da, indicating the SOD1 oxidized form. Analysis of SOD1 peaks in lymphoblasts of both patients and controls, showed a pattern of PTMs similar to hSOD1. However differences between patients and controls were observed. In patients, the oxidized SOD1 peak was increased, indicating more oxidized SOD1 in sALS lymphoblast. In untreated SH cells, decomposition of SOD1 peak was similar to healthy control lymphoblasts and hSOD1, whereas in cells undergoing oxidative stress, analysis of SOD1 peak showed an increase of SOD1 oxidized form as observed in sALS patients and hSOD1 treated with hydrogen peroxide.

Discussion: These data suggest that in sALS patients a large portion of SOD1 is oxidized demonstrating the hypothesis of increased oxidized WT-SOD1 in sALS cells. This alteration could confer to WT protein, toxic and pathogenic properties of ALS-linked mutant SOD1, as previously suggested (1). Data on treated SH-SY5Y confirm the validity of SOD1 oxidation peak. Moreover the innovative bioinformatic analysis of peak decomposition proved to be a useful tool in proteomic analysis with SELDI-TOF-MS.

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P88 MISFOLDED SOD1 IN CEREBROSPINAL FLUID FROM ALS PATIENTS

ZETTERSTRÖM P[1], ANDERSEN PM[2], BRÄNNSTRÖM T[1], MARKLUND SL[1]

[1]*Umeå Universtity, Medical Biosciences, Umeå, Sweden,* [2]*Umeå Universtity, Pharmacology and Clinical Neuroscience, Umeå, Sweden*

E-mail address for correspondence: per.zetterstrom@medbio.umu.se

Keywords: misfolded SOD1, cerebrospinal fluid, interstitial space

Background: It is still not known how mutant SOD1 is toxic to motor neurons or how the disease is spread throughout the motor system. The SOD1 species toxic to motor neurons remain elusive with the dominating hypothesis being that they are un/misfolded SOD1. Only a misfolded conformation could be common to all the over 150 different SOD1 mutations known today. Therefore, a misfolded SOD1 species could exert a mechanism common for all mutants. Recent data shows that SOD1 can be secreted extracellularly, the mechanism is still unknown but may involve chromogranins A and B binding misfolded SOD1 and transporting it out of the cell. Extracellular SOD1 is toxic to primary motor neuron cultures and can cause gliosis and other kinds of non-cell autonomous damage. Therapeutic strategies involving active and passive immunization against SOD1 in mice have also shown promising results arguing for an extracellular SOD1 factor. That SOD1 exists in the cerebrospinal fluid (CSF) is well known, but nothing is known about misfolded SOD1 in CSF.

Objectives: The objective of this study was to develop an ELISA method to be able to measure the levels of misfolded SOD1 in CSF samples from ALS patients (11 SOD1 mutation carriers, 9 non-SOD1 fALS, 31 sALS, total n=51) and neurological controls (n=52).

Methods: We have created a set of anti-peptide antibodies that are highly specific for misfolded SOD1. Three of these antibodies, towards amino acids 23-39, 57-72 and 111-127 in human SOD1 respectively, were used to set up three different ELISAs highly specific for misfolded SOD1 (misELISA). The secondary antibody used in the misELISAs has 8-fold larger reactivity towards misfolded SOD1 than native SOD1. We have also measured total amounts of SOD1 and total protein levels in the CSF samples.

Results: We have for the first time been able to demonstrate and quantify misfolded SOD1 in CSF samples. The concentrations are very low, only about 1/2,000 of the SOD1 molecules in CSF are misfolded. We found no significant difference in contents of misfolded SOD1 between controls and ALS cases with any of the three misELISAs. When corrected for amounts of total SOD1 there were still no differences, nor did we find any differences between sALS patients and fALS patients with and without SOD1 mutations.

Discussion: The low levels of misfolded SOD1 found in the CSF argue against a direct role of misfolded SOD1 in pathogenesis of ALS. The concentrations are a thousand times lower than the concentrations toxic to cultured primary motor neurons. Even low concentrations of extracellular misfolded SOD1 could trigger non-cell autonomous effects and/or function as a seed in a prion-like process leading to protein aggregation and thereby be part of spreading the disease in the motor areas.

P89 MISFOLDED SOD1 IS A CHARACTERISTIC OF BOTH FAMILIAL AND SPORADIC FORMS OF ALS

GRAD L[1], MACKENZIE I[2], O'NEILL M[1], CASHMAN N[1]

[1]*Brain Research Centre, Department of Medicine,* [2]*Department of Pathology and Laboratory Medicine; University of British Columbia, Vancouver, BC, Canada*

E-mail address for correspondence: lgrad@interchange.ubc.ca

Keywords: superoxide dismutase, protein misfolding, antibodies

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the progressive death of motor neurons. The sporadic form of the disease (SALS) makes up ~90% of all cases of ALS, with the remainder being an inheritable, or familial, form (FALS). 10-20% of all FALS cases are due to mutations in superoxide dismutase 1 (SOD1), a soluble ubiquitously-expressed free-radical defense enzyme. Mutations in SOD1 promote misfolding and/or aberrant oxidation of the protein, leading to its subsequent aggregation and resulting in a cytotoxic gain-of-function. There is increasing evidence that oxidation/misfolding of wild-type SOD1 acquires many of the same cytotoxic properties as mutant SOD1, suggesting mutation in SOD1 is not essential for its participation in ALS pathogenesis.

Objective: To determine if misfolded SOD1 participates in a common pathological mechanism in both sporadic and familial forms of ALS.

Methods: To address the role of misfolded SOD1 in different forms of ALS, we utilized monoclonal antibodies that specifically recognize misfolded/oxidized forms of SOD1 to test for their presence in human spinal cord from SOD1-FALS and non-SOD1 SALS patients. These disease-specific epitope (DSE) antibodies were used in immunoprecipitation and immunohistochemistry of human ALS spinal cord tissue. We assessed SOD1 misfolding by protease sensitivity, as native SOD1 is highly resistant to digestion. We further analyzed FALS/SALS spinal cord tissue by non-reducing electrophoresis and immunoblotting to detect other biochemical similarities.

Results: Our results confirm the presence of misfolded/oxidized SOD1 in spinal cords from both FALS and SALS by both immunoprecipitation and immunohistochemistry, using our DSE antibodies. This misfolded SOD1 is highly protease-sensitive, suggesting a structural loosening of the enzyme from its normally protease-resistant conformation. We also detect a significant increase in the abundance of disulfide cross-linked SOD1-containng hetero-oligomers in both FALS and SALS spinal cords, further confirming the presence of misfolded/oxidized SOD1. In addition, high molecular weight SOD1-containing species are associated with a nitrotyrosine signature, suggesting pathological SOD1 is in complex with a protein other than itself, and advanced glycation end-products, post-translational modifications associated with oxidative stress and progression of age-related neurodegenerative disease.

Discussion: The clinical presentation of ALS shows similarities between SOD1-related FALS and typical SALS suggesting a possible common mechanism for both. Moreover, there is increasing evidence in the literature implicating a role for SOD1 in non-SOD1 SALS; other studies have found common molecular signatures of SOD1 in cases of FALS and SALS. The evidence is consistent with the hypothesis that aberrant oxidation and protein misfolding of SOD1 is a primary molecular mechanism common to all forms of ALS.

Conclusions: We conclude that misfolded/oxidized SOD1 is a pathological characteristic in both SOD1-related FALS and non-SOD1 SALS and likely participates in a pathogenic mechanism common to both types of ALS.

P90 JUVENILE AMYOTROPHIC LATERAL SCLEROSIS WITH FUS/TLS PATHOLOGY AND MUTATION

HUANG E[1], ZHANG J[1], GESER F[2], TROJANOWSKI J[2], STROBER J[1], DICKSON D[3], BROWN R[4], SHAPIRO B[5], LOMEN-HOERTH C[1]

[1]*University of California San Francisco, San Francisco, CA, United States,* [2]*University of Pennsylvania, Philadelphia, PA, United States,* [3]*Mayo Clinic, Jacksonville, FL, United States,* [4]*University of Massachusetts, Worcester, MA, United States,* [5]*Case Western Reserve University, Cleveland, OH, United States*

E-mail address for correspondence: eric.huang2@ucsf.edu

Keywords: FUS/TLS, mutation, pathology

Background: Juvenile amyotrophic lateral sclerosis (ALS) with basophilic inclusions is a well-recognized entity. However, the molecular underpinnings of this devastating disease are poorly understood.

Objectives: The goal of this study is to provide a comprehensive genetic and neuropathological characterizations in two young women with fatal rapidly progressive ALS with basophilic inclusions.

Methods: Immunohistochemistry, immunogold electron microscopy and analyses of the FUS/TLS (fused in sarcoma/translocated in liposarcoma) gene to identify potential mutations.

Results: A germline mutation (P525L) was detected in the FUS/TLS gene in one of the two patients. Postmortem examinations in both cases revealed severe loss of spinal motor neurons with remaining neurons showing basophilic inclusions that contain abnormal aggregates of FUS proteins and disorganized intracellular organelles, including mitochondria and endoplasmic reticulum. In both patients, the FUS-positive inclusions were also detected in neurons in layers IV-V of cerebral cortex and several brainstem nuclei. In contrast, spinal motor neurons in patients with late-onset sporadic ALS showed no evidence of abnormal accumulation of FUS protein.

Discussion: These results underscore the importance of FUS mutations and pathology in rapidly progressive juvenile ALS. Furthermore, our study represents the first detailed characterizations of neuropathological findings in rapidly progressive juvenile ALS patients with a mutation in the FUS/TLS gene.

Conclusions: Our results indicate that FUS/TLS mutation and FUS pathology are important part of the work-up for juvenile ALS with basophilic inclusions.

P91 ABNORMAL TDP-43 AND FUS PROTEINS IN MUSCLES OF SPORADIC IBM: SIMILARITIES IN A TARDBP-LINKED ALS PATIENT

MILLECAMPS S[1], LAIN AH[2], DUBOURG O[3], SALACHAS F[4], BRUNETEAU G[4], LEGUERN E[1], SEILHEAN D[3], DUYCKAERTS C[3], MALLET J[1], MEININGER V[4], PRADAT P-F[4]

[1]*Research Center of the Brain and Spinal Cord Institute, Pitié-Salpétrière Hospital, Paris, France,* [2]*Department of Neuropathology, Madrid, Spain,* [3]*Department of Neuropathology,* [4]*Department of Neurology, Pitié-Salpétrière Hospital, Paris, France*

E-mail address for correspondence: pierre-francois.pradat@psl.aphp.fr

Keywords: inclusion body myositis, TDP-43, FUS

Background: TAR DNA-binding protein (TDP-43) and FUS (Fused in Sarcoma) are two components of protein aggregates in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Both proteins are ubiquitously expressed and share striking functional and structural homology. TDP-43 aggregates have also been described in muscles of sporadic inclusion body myositis (s-IBM) patients, but whether this is associated with FUS abnormalities remains unknown.

Objective: We studied TDP-43 and FUS expression in patients with s-IBM and ALS.

Results: Protein levels of TDP-43 and FUS were evaluated in muscle biopsies of five patients with s-IBM, five patients with sporadic ALS (SALS) (including one patient with *TARDBP* gene mutation) and five controls with myalgia. All s-IBM patients showed abnormalities on immunoblots for TDP-43, in particular accumulation of c-terminal truncated TDP-43 fragments. In these patients, there was also a consistent decrease in FUS protein levels with a concomitant increase of a high molecular weight FUS immunoreactive protein. Interestingly, the *TARDBP*-linked ALS patient showed very similar abnormalities in contrast to the 4 SALS cases which were indistinguishable from controls. Sarcoplasmic TDP-43 inclusions, and loss of nuclear TDP-43 protein were observed only in muscles of s-IBM patients but neither in SALS nor in *TARDBP*-linked ALS patients.

Discussion and conclusion: s-IBM is associated with abnormalities in TDP43 immunoreactivity and loss of normal FUS in muscle. Similar mechanisms might be at work in *TARDBP*- linked ALS.

P92 CLOSE ASSOCIATION OF TDP-43 PATHOLOGY WITH LOSS OF RNA EDITING ENZYME ADAR2 IN MOTOR NEURONS IN SPORADIC ALS

AIZAWA H[2], SAWADA J[2], HIDEYAMA T[1], YAMASHITA T[1], KWAK S[1]

[1]*Graduate School of Medicine, University of Tokyo, Tokyo, Japan,* [2]*Asahikawa Medical College, Asahikawa, Japan*

E-mail address for correspondence: kwak-tky@umin.net

Keywords: TDP-43, ADAR2, AMPA receptor

Background: Motor neurons of normal subjects express GluR2, a subunit of the AMPA receptor, with arginine (R) at the Q/R site, whereas considerable proportion of those of sporadic ALS patients express GluR2 with glutamine (Q) at the Q/R site. Conversion of Q (CAG) to R (CGG) at the Q/R site of GluR2 pre-mRNA is specifically catalyzed by adenosine deaminase acting on RNA 2 (ADAR2). Therefore, it is likely that ADAR2 activity is not sufficient to edit this site of all the GluR2 mRNAs in motor neurons of sporadic ALS. Because failure to edit the Q/R site of all the GluR2 is a direct cause of death of motor neurons, we have proposed that GluR2 Q/R site-underediting due to ADAR2 underactivity is a neuronal death-inducing cause in sporadic ALS. Formation of TDP-43-positive inclusion bodies with loss of TDP-43 in the nucleus is another disease-specific molecular abnormality in sporadic ALS motor neurons.

Objectives: To investigate whether there is a molecular link between reduced ADAR2 activity and TDP-43 pathology, we immunohistochemically investigated localization of ADAR2 and phosphorylated TDP-43 in the motor neurons of sporadic ALS.

Methods: This study was conducted using lumbar spinal cords from seven cases of sporadic ALS and six disease-free control cases. Western blot analysis was performed on nuclear and cytoplasmic fractions of human brains. Formalin-fixed paraffin-embedded sections were double-immunostained with anti-ADAR2, anti-TDP-43 and anti-phosphorylated TDP-43 antibodies. In addition, effects of paraffin-embedding and postmortem delay on the immunoreactivity was tested.

Results: ADAR2a and ADAR2b were localized predominantly in the nucleus in Western blot analysis and immunohistochemical examination of frozen spinal cords. The number of anterior horn motor neurons (AHCs) was reduced to 40% of that in control cases ($P < 0.0001$). We found that all AHCs examined ($n = 380$) were ADAR2-positive in the control cases, whereas more than half of them were ADAR2-negative in each of the ALS cases examined. All ADAR2-negative neurons ($n = 98$ out of 170 AHCs in ALS cases) had cytoplasmic inclusions that were immunoreactive to phosphorylated TDP-43 but lacked non-phosphorylated TDP-43 in the nucleus.

Discussion and conclusions: Our results indicate a molecular link between reduced ADAR2 activity and TDP-43 pathology in motor neurons of sporadic ALS. Because TDP-43 knockout mice are embryonic lethal, and because motor neurons undergo slow death in conditional ADAR2 knockout mice, it is likely that either one of the two ALS-associated abnormalities induces neuronal death via causing the other, rather than both of them occur simultaneously as a result of other upstream abnormalities.

P93 REGULATION OF TDP-43 BY NR2A-CONTAINING NMDA RECEPTOR/PTEN SIGNALING: ITS ROLE IN GLUTAMATE TOXICITY-INDUCED NEURONAL INJURY

ZHENG M[1,2], WAN Q[2], FAN D[1]

[1]*Peking University Third Hospital, Beijing, China,* [2]*University of Nevada School of Medicine, Reno, United States*

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: TDP-43, PTEN, glutamate injury

Background and objectives: Two DNA/RNA binding proteins TDP-43 (TAR DNA binding protein) and FUS/TLS (fused in sarcoma/translocation in liposarcoma) were recently found to be hallmarks of amyotrophic lateral sclerosis (ALS). Abnormal TDP-43 and FUS/TLS comprised pathological inclusions which extensively distributed in sporadic ALS (SALS) and non-SOD1 mutation familial ALS (FALS). Both of them played an important role in RNA processing, which implied the high metabolic demand of motor neurons, placed great stress on transcription leading to preferential vulnerability of this cell type. Enhanced transcription activity might be a pro-survival response of motor neurons to environmental injury. Here we demonstrated that glutamate neurotoxicity increased TDP-43 expression, and this enhanced expression was negatively regulated by PTEN.

Methods: Downregulation of PTEN expression induced by glutamate injury could be subdued by inhibition of NMDAR activity, which implicated NMDAR activity involved in regulation of PTEN expression.

Results: Application of different NMDAR subunits antagonist showed that inhibition of NR2AR activity could decrease PTEN downregulation and TDP-43 upregulation induced by glutamate injury. Inhibition of NR2BR, however, had little influence on PTEN and TDP-43 expression. Comparing with enhanced expression of TDP-43 by inhibition of PTEN alone, inhibition of NR2AR and PTEN together did not prominently decrease this upregulation of TDP-43 expression, which indicated that NR2AR and PTEN were in the same intercellular pathway, and NR2AR located at the upstream of PTEN to regulate TDP-43 expression. We also tested the effect of TDP-43 on neuron injury induced by glutamate neurotoxicity. Result showed that knockdown of TDP-43 expression promoted glutamate induced neuronal death and TDP-43 overexpression provided a protective effect to damaged neurons.

Conclusions: Consistent with neurotoxicity early in the disease process, glutamate injury upregulates TDP-43 expression through NR2A-containing NMDA receptor/PTEN signaling, and increased TDP-43 protects neurons from glutamate toxicity-induced injury. In early stage of ALS, activation of the NR2AR/PTEN/TDP-43 pro-survival pathway in glutamate induced neuron injury might be a prospective therapeutic target.

P94 HFE H63D CONTRIBUTES TO INCREASED TDP-43 LEVEL AND CYTOSOLIC LOCALIZATION

LIU Y[1], SIMMONS Z[2], CONNOR J[1]

[1]*Department of Neurosurgery,* [2]*Department of Neurology; Penn State University, M.S. Hershey Medical Center, Hershey, PA, United States*

E-mail address for correspondence: yliu2@hmc.psu.edu

Keywords: HFE, TDP-43, iron

Background: Genetic studies on different populations have reported an increased frequency of a specific form of HFE polymorphism, H63D, in individuals with amyotrophic lateral sclerosis (ALS). Mutation of the HFE gene is best known as being associated with cellular iron overload, but the mechanism by which H63D HFE might increase the risk of motor neuron degeneration is unknown. The TAR DNA binding protein 43 (TDP-43) is present in neuronal inclusions in ALS, as well as in frontotemporal dementia. In ALS, the expression of TDP-43 is up-regulated and in contrast to its normal nuclear localization, becomes cytosolic. Several TDP-43 mutations have been discovered that may increase TDP-43 aggregation and promote neuronal toxicity. However, in most ALS cases without TDP-43 mutations, the factors that transform the wild type (WT) endogenous TDP-43 into its pathological form remain elusive. In this study, we sought to examine the link between HFE H63D and TDP-43 aggregation.

Objective: To determine whether and how the presence of the HFE H63D mutation, a putative risk factor for ALS, contributes to the up-regulation and cytoplasmic aggregation of TDP-43.

Methods: We assessed the expression and localization of TDP-43 in two models: a cell culture model expressing inducible HFE wild type (WT) or H63D protein and a knock-in mouse model, carrying the H67D variant of HFE which is the mouse analogue of the H63D mutation.

Results: Expression of WT HFE protein decreased TDP-43 expression. In contrast, the presence of the iron overload mutant HFE H63D was associated with an up-regulation of TDP-43 expression. Moreover, cytoplasmic localization of TDP-43 was detected in HFE H63D expressing cells. Iron challenge in both HFE WT and H63D expressing cells resulted in an increase of TDP-43. Treatment with desferroxamine mesylate (DFO), an iron chelator, did not completely reverse the increased TDP-43 associated with HFE H63D, or change the TDP-43 expression in the WT HFE cells. The relationship between HFE H63D and TDP-43 in the cell culture model was also found in an *in vivo* model. In the lumbar spinal cord homogenates from HFE H67D mice at age 6-months, there were elevated levels of TDP-43 expression compared to the WT littermates. At 12-months of age, the difference in TDP-43 expression between the WT and H67D mice was increased.

Discussion and conclusions: These data strongly support our hypothesis that HFE H63D contributes to the abnormal expression and localization of TDP-43. Increased intracellular iron might be the mechanism that underlies this association. HFE H63D and an associated increase in iron may transform the WT endogenous TDP-43 into pathological aggregates in ALS patients without TDP-43 mutations.

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THEME 5 GENETICS

P95 H63D POLYMORPHISM IN THE HEMOCHROMATOSIS GENE IS ASSOCIATED WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS IN CHINA

XU Y, HE X, YAO X

West China Hospital, Sichuan University, Chengdu, Sichuan Province, China

E-mail address for correspondence: neuroxym@163.com

Keywords: HFE gene, single nucleotide polymorphism

Background and purpose: H63D(His63Asp) polymorphism in HFE gene has been reported as a risk factor for amyotrophic lateral sclerosis (ALS) in Europe and America, whereas no data has been obtained for Asia. Herein, we investigated the frequency of this polymorphism in a Chinese population.

Methods: A total of 195 individuals with sporadic ALS (sALS) from three centers in China and 405 unrelated healthy controls were recruited. The HFE H63D polymorphism was determined by restriction fragment length polymorphism (RFLP) analysis.

Results: Sporadic ALS was significantly related to H63D polymorphism in heterozygous carriers (odds ratio 3.10, CI: 1.49 to 6.47, P=0.002).

Conclusions: The HFE H63D polymorphism may contribute to the development of sporadic ALS in China.

P96 TWO NOVEL ANGIOGENIN (ANG) GENE MUTATIONS AND THEIR PATHOGENESIS

AKIMOTO C[1], MORITA M[1], SAKASHITA E[2], ENDO H[2], NAKANO I[1]

[1]*Neurology, Shimotsuke, Tochigi, Japan,* [2]*Biochemistry, Shimotsuke, Tochigi, Japan*

E-mail address for correspondence: ckawamata@mac.com

Keywords: angiogenin, V105A, P (-4) Q

Background: Mutations of *ANG* have been found in ALS, and recent studies revealed angiogenin protects motoneurons from hypoxic injury.

Objectives: We screened the ANG gene in sporadic ALS cases, and examined synthesis of mutated angiogenin protein in HeLa cells.

Methods: Screening of the ANG gene: We scanned the coding regions of the ANG gene by high resolution melting (HRM) analysis and sequenced the samples indicated to include mutations. Synthesis of ANG protein: We constructed mammalian expression plasmid vectors with C terminal FLAG tags including wild and mutated type ANG genes. The resultant plasmid DNAs were transfected into HeLa cells, and then angiogenin protein synthesis was examined by Western blotting and immunocytochemistry.

Results: We identified two novel mutations (V105A and P (-4) Q) in 184 sporadic ALS patients. One was present in the mature protein region, and the other in the signal peptide sequence. Based on the results of expression vector, we verified the mature size angiogenin protein was synthesized with the P (-4) Q mutated ANG gene (mutated type), but the early secretion was reduced compared with the wild type. Both the mutated and wild type angiogenin proteins were localized to the endoplasmic reticulum (ER) and Golgi apparatus, but transportation of the mutated type from the ER to the Golgi apparatus was slightly obstructed. We speculate that it is one of the reasons for the delayed secretion.

Discussion and conclusion: The prevalence of the *ANG* mutation in sporadic ALS was 1.08% in our Japanese group, which was almost the same (0.3~1.0%) as in European countries. In the case of an angiogenin synthesis state like the hypoxic one, the early secretion of the P (-4) Q protein is reduced with the consequent increase in neuronal damage. Judging from our experiment, the mutation in the signal peptide sequence in the ANG gene could be pathogenic.

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P97 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL SOD1 SPLICE SITE MUTATION ASSOCIATED WITH FAMILIAL ALS

BIRVE A[1], NEUWIRTH C[3], WEBER M[3], MARKLUND S[2], NILSSON A-C[1], JONSSON A[2], ANDERSSON R[1], ANDERSEN P[1]

[1]*Department of Pharmacology and Clinical Neuroscience, Umea, Sweden,* [2]*Department of Medical Biosciences, Umea, Sweden,* [3]*Neuromuscular Diseases Unit, St. Gallen, Switzerland*

E-mail address for correspondence: anna.birve@neuro.umu.se

Keywords: SOD1, intronic mutation, splice variant

Background: More than 145 mutations have been found in the gene *CuZn-Superoxide dismutase* (*SOD1*) in patients with amyotrophic lateral sclerosis (ALS). The vast majority are single nucleotide substitutions in the coding region causing missense mutations. Most mutations result in a protein with reduced dismutation. Four intronic mutations have been associated with ALS previously, all in intron 4. In this study we report the identification and characterization of a novel intronic mutation in intron 3 found in a Swiss patient with familial ALS.

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Objectives: The purpose of this study was to identify the suspected *SOD1* mutation in a 42-year-old FALS patient with significantly low SOD1 enzymatic activity in erythrocytes. The patient was DNA-sequence analysed in *SOD1* but there was no sequence aberration found in the coding regions. This called for a further investigation of the *SOD1* gene.

Methods: The *SOD1* gene of the FALS patient was analysed with DNA sequencing and Reverse Transcription-PCR. Interned based splicing algorithm tools were used to find potential splice site mutations. SOD1 activity was measured and Western immunoblotting was used to examine the presence and characteristics of SOD1 proteins.

Results: Sequence analysis revealed that the patient was heterozygous for a thymine to guanine mutation 7 bp upstream of exon 4 (c.240–7T>G). Splicing analysis tools revealed a potential novel splice site that would add 6 bp to the mRNA. This mRNA would insert Ser and Ile between Glu78 and Arg79 in the SOD1 protein (SOD1 E78_R79insSI). Both the predicted mutant transcript and the mutant protein were found to be highly expressed in fibroblasts from the patient and the SOD activity was approximately normal in these cells.

Discussion: The two mRNAs, wild type and mutant, as well their respective proteins appeared to be expressed in equal amounts. These findings show that the usage of the alternative splice site must be near 100%. Comparison of SOD1 protein and activity analyses in fibroblasts suggests that, despite the insertion of two novel amino acids into the metal ion binding loop IV, the mutant SOD1 has roughly normal enzymatic activity, close to native structure and is relatively stable. However, since the enzymic activity in erythrocytes was ~50% of controls it seems the mutant protein shows the reduced stability typical of ALS-linked mutant SOD1s.

Conclusion: The investigation suggests that the intronic mutation found in the FALS patient is causing the disease, and highlights the importance of wide exon flanking sequencing and transcript analysis combined with erythrocyte SOD activity analysis in comprehensive search for *SOD1* mutations in ALS cases.

P98 L67P: A NOVEL EXON 3 SOD1 MUTATION IN AN ITALIAN ALS PATIENT

DEL GRANDE A[1], LUIGETTI M[1], CONTE A[1], MADIA F[2], MANCUSO I[3], LATTANTE S[3], MARANGI G[3], STIPA G[4], TONALI PA[1], ZOLLINO M[3], SABATELLI M[1]

[1]*Catholic University, Department of Neurosciences, Institute of Neurology, Rome, Italy,* [2]*Complesso Integrato Columbus, Rome, Italy,* [3]*Catholic University, Institute of Human Genetics, Rome, Italy,* [4]*Azienda ospedaliera S.Maria, servizio di Neurofisiopatologia, Terni, Italy*

E-mail address for correspondence: msabatelli@rm.unicatt.it

Keywords: SOD1 mutation

Background: Mutations in SOD1 gene are responsible for 20% of familial ALS and more than 130 mutations have been identified so far. The majority of them are located in exons 4 and 5, less than one third are in exons 1 and 2, while only eight mutations (5%) have been reported among the 24 codons of exon 3, generally disclosing low penetrance and predominant lower motor neuron involvement.

Objectives: To analyze clinical and electrophysiological features of a patient affected by sporadic ALS bearing a novel mutation in exon 3 of the Cu/Zn superoxide dismutase gene.

Methods: After obtaining written informed consent from the patient and her parents, all five exons and flanking intron regions of the *SOD1* gene were analyzed using direct genome sequencing.

Results:: We describe a novel L67P mutation located in exon 3 of the Cu/Zn superoxide dismutase gene. The propositus was a 38 year old woman affected by slowly progressive ALS with onset at the age of 36 years and disclosing pure lower motor neuron signs. Motor Evoked Potentials were within normal limit. At clinical examination she was able to walk with a cane and presented moderate wasting and weakness of all four limbs, more evident in the distal muscles and in lower limbs. There were no bulbar signs and respiratory function was normal. There were no other cases of ALS in her family but the mutation was found also in her healthy father, aged 76.

Discussion and conclusions: A total of 9 mutations involving exon 3 of SOD1 have been described, including the present one. Our data confirms that variable penetrance and predominant lower motor neuron involvement are common features in patients bearing mutations in exon 3 of SOD1.

P99 PHENOTYPIC PRESENTATION OF I113T SOD1 ALS

DONKERVOORT S, AJROUD-DRISS S, SIDDIQUE N, SIDDIQUE T

Northwestern University, Chicago, IL, United States

E-mail address for correspondence: s-donkervoort@northwestern.edu

Keywords: SOD1, I113T, FALS

Over 140 disease causing mutations have been reported in Cu/Zn superoxide dismutase (SOD1). The I113T mutation has been reported in both familial amyotrophic lateral sclerosis (FALS) and apparently sporadic amyotrophic lateral sclerosis (SALS). Genetic counseling for I113T SOD1 ALS is challenging due to its reported reduced penetrance, as well as variability in disease onset and progress. I113T is one of the most common SOD1 mutations; however, published literature is limited to individual case reports. We present clinical data on the largest cohort of I113T SOD1 patients to date, comprised of 28 ALS patients from 18 different kindreds, as well as a comprehensive literature review. Our data illustrates wide variability in age of onset (ranging from 23 to 77 years) and disease duration (5 to 145 months). Extensive inter- and intra-familial phenotypical variability was observed. None of the I113T SOD1 ALS patients had frontotemporal dementia and/or apparently sporadic disease, although reduced penetrance was observed in 3 families with obligate carriers of 66, 77 and, 91 years of age. Interestingly, because of the atypical slow presentation in some patients, 4 patients were initially diagnosed with neuropathy, 1 with multiple sclerosis and 1 with asthma. This analysis presents the phenotypic expressions of this common SOD1 mutation, which will aid in providing appropriate genetic counseling and testing for patients and families with FALS.

P100 TARDBP GENE MUTATIONS AMONG CHINESE PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

HUANG R, FANG D-F, MA M-Y, GUO X-Y, ZHAO B, ZENG Y, ZHOU D, YANG Y, SHANG H-F

Department of Neurology, West China Hospital of Sichuan University, Chengdu, Sichuan, China

E-mail address for correspondence: hfshang@yahoo.com

Keywords: TARDBP gene, mutation

Background: Recently, several *TARDBP* mutations have been identified in sporadic amyotrophic lateral sclerosis

(SALS) patients of different ethnicity. No *TARDBP* mutation analysis among Chinese SALS patients has been reported.

Objective: This study aims to analyze the clinical features and mutations in the *TARDBP* gene among Chinese patients with SALS.

Methods: The clinical characteristics of patients diagnosed with adult-onset SALS were analyzed. The frequencies of *TARDBP* mutations and the association between these mutations and the clinical features of ALS were analyzed.

Results: One hundred and sixty-five patients were studied. The mean age of onset was 50.8±12.0 years. The mean diagnostic delay was 18.8±17.1 months. A novel missense mutation (p.N378S) and a novel silent change (p.A321A) were detected in two male patients, respectively. A new variant of c.1098C>G in exon 6 and two reported variants, g. IVS1+85C>T in intron 1 and c.57A>G in exon 2, were found. The frequency of the 'G' variant of c.57A>G in exon 2 and the 'G' variant of c.1098C>G in exon 6 were significantly lower in the patient group than in the control ($P<0.001$ and $P=0.024$, respectively).

Conclusions: To the best of our knowledge, this is the first study on *TARDBP* mutation among Chinese patients with SALS. Our findings provide evidence that the frequency of *TARDBP* gene mutations is rare among Chinese SALS patients (0.61%). Several polymorphisms may influence susceptibility to ALS.

P101 PHENOTYPES IN SWISS PATIENTS WITH FAMILIAL AND SPORADIC ALS CARRYING TARDP MUTATIONS

WEBER M[1], MORITA M[2], NEUWIRTH C[1], TIERBACH J[3], PERREN F[4], ANDERSEN P[5]

[1]*Neuromuscular Diseases Unit, Kantonsspital, St. Gallen, Switzerland,* [2]*Department of Neurology, Jichi Medical University, Yakushiji, Shimotsuke-shi, Tochigi, Japan,* [3]*Blood Collection Centre, Kantonsspital, St.Gallen, Switzerland,* [4]*Department of Neurology, University Hospital, Geneva, Switzerland,* [5]*Department of Neurology, University Hospital, Umeå, Sweden*

E-mail address for correspondence: christoph.neuwirth@kssg.ch

Keywords: TARDP mutations, phenotypes, Switzerland

Background: Recently mutations in the TARDP gene which codes for the TAR DNA binding protein 43 (TDP-43) have been identified in familial and sporadic ALS patients. The frequency of TARDP mutations seems different in various European populations and phenotypic variability is high.

Objective: To further define the phenotypic spectrum of TARDP mutations and their frequency in a European population.

Methods: A total of 246 patients diagnosed with ALS (221 sporadic cases, 43 FALS cases in 25 families) were screened for TARDP mutations. Except for one patient who was followed at the University of Geneva all patients were followed at the Kantonsspital St.Gallen. FALS cases carrying SOD mutations were excluded.

Results: In 4 patients TARDP mutations were identified. Two female ALS patients, one apparently sporadic and one familial case, carried the Asn352Ser mutation. Both had limb onset and a slowly progressive course of the disease. In the sporadic case with a survival of 8 years, bulbar muscles were spared until death despite severe respiratory insufficiency. The other patient died 7 years after disease onset. In a 44-year old female patient a novel mutation (Gly376Asp) was identified. Family history suggested an autosomal dominant trait with complete penetrance. Survival was 20 months in her and only 6 to 18 months (mean 13 months) in relatives. A fourth male case carried the Ala90Val mutation. None of the patients had cognitive impairment. The frequency of TARDP mutations was 1% in SALS and 9% in FALS.

Conclusion: The frequency of TARDP mutations in the Swiss population is higher compared to other European populations. The novel Gly376Asp mutation is associated with rapid disease progression while the Asn352Ser mutation is associated with slow disease progression.

P102 HOMOZYGOUS A382T MUTATION OF TARDBP GENE IN AN ITALIAN PATIENT WITH AN ATYPICAL ALS PHENOTYPE

MORELLI C, TILOCA C, COLOMBRITA C, TICOZZI N, DORETTI A, MESSINA S, RATTI A, SILANI V

Department of Neurology - Università degli Studi di Milano - IRCCS Istituto Auxologico Italiano, Milan, Italy

E-mail address for correspondence: claudiamore@tiscali.it

Keywords: TARDBP, TDP-43, genetics

Background: A 44-year-old woman of Southern Italian origin, affected by ALS with bulbar onset and without familial history of motor neuron disease, was admitted to our ALS Centre. The neurological examination revealed upper and lower motor neuron signs in the bulbar and cervical regions and only upper motor neuron signs in the lumbosacral region (probable ALS according to El Escorial criteria). Electromyography showed active denervation in the right first dorsal interosseous muscle and chronic denervation in the left one. Cerebral MRI revealed periventricular and subcortical demyelinating lesions, without pathologic enhancement after gadolinium administration. Cervical MRI was normal. Numerous oligoclonal bands restricted to the cerebrospinal fluid were detected by isoelectrofocusing. Search for serum anti-HIV1/2 and anti-Borrelia burgdorferi antibodies was negative. A thrombophilic condition was excluded. Thus neuroradiological and laboratory tests suggested the presence of an inflammatory demyelinating disease of the CNS, in addition to features characteristic of ALS.

Objectives: We investigated the potential genetic contribution to this atypical ALS case with a demyelinating disease of the CNS by performing a mutational screening of the ALS causative genes *SOD1*, *ANG*, *TARDBP* and *FUS*.

Methods: After informed consent, blood was withdrawn and DNA was extracted according to standard procedures. Molecular analyses of *SOD1*, *ANG*, *TARDBP* and *FUS* genes were conducted by direct DNA sequencing. Array CGH (Comparative Genomic Hybridization) analysis was performed on the Agilent platform.

Results: Mutations in *SOD1*, *ANG* and *FUS* genes were excluded in our patient. Molecular analysis of *TARDBP* gene led to the identification of the homozygous missense mutation A382T (1144G>A) in exon 6. Furthermore, array-CGH analysis provided no evidence for deletions of the *TARDBP* genomic region.

Discussion and conclusion: Mutations in *TARDBP* gene account for 5% of familial ALS and 2% of sporadic ALS cases. Among the *TARDBP* gene mutations, the A382T variant represents the most frequent one, but to our knowledge this

is the first report of an ALS patient carrying this mutation in an homozygous state.
We speculate that a dose-dependent mechanism could explain this peculiar phenotype. Absence of wild-type TDP-43 with loss of its physiological nuclear function or a double dose of the mutant protein could be related to the development of ALS complicated by a demyelinating process. This hypothesis could also be supported by determination of TDP-43 levels in the CSF of our patient, compared with those of patients carrying heterozygous or no *TARDBP* mutations.

P103 MULTIPLE SYSTEM DEGENERATION WITH BASOPHILIC INCLUSIONS IN JAPANESE ALS PATIENTS WITH FUS MUTATION

TATEISHI T[1], HOKONOHARA T[1,2], KIKUCHI H[1], MIURA S[4], OHYAGI Y[1], IWAKI T[3], FUKUMAKI Y[2], KIRA J-I[1]

[1]*Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan,* [2]*Division of Human Molecular Genetics, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Fukuoka, Japan,* [3]*Department of Neuropathology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan,* [4]*Division of Respirology, Neurology and Rheumatology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan*

E-mail address for correspondence: tatetaka@neuro.med.kyushu-u.ac.jp

Keywords: fused in sarcoma/translated in liposarcoma, basophilic inclusion, glial cytoplasmic inclusion

Background: Approximately, 5–10% of cases are familial forms, which are usually transmitted by an autosomal dominant trait. Recently, Kwiatkowski and Vance reported mutations in a gene encoding another DNA/ RNA-binding protein called fused in sarcoma (FUS) specific for familial ALS.

Objectives: To clarify the clinical and pathological findings in a large Japanese familial ALS pedigree with an autosomal dominant inheritance pattern, and carrying a *FUS* R521C mutation.

Methods: We carried out clinical, neuropathological and genetic studies on a large pedigree with familial ALS. In six successive generations of this family, 16 individuals of both sexes were affected by progressive muscle atrophy and weakness, indicating an autosomal dominant trait.

Results: Both sexes (11 men and 5 women) were affected. Mean age at onset was 40.6±13.8 years, and mean duration from onset to respiratory failure was 11.7±7.3 months. Three out of six patients examined, showed preferential involvement of the proximal upper extremities with flailing arms and subsequent spread of motor weakness to the lower extremities. Motor paralysis progressed rapidly in these patients, culminating in respiratory failure within 1 year. The missense mutation c.1561 C>T; p.R521C was found in exon 15 of *FUS* in the four patients examined. Neuropathological study of one autopsied case with the FUS mutation revealed multiple system degeneration in addition to upper and lower motor neuron involvement: the globus pallidus, thalamus, substantia nigra, cerebellum, inferior olivary nucleus, solitary nucleus, intermediolateral horn, Clarke's column, Onuf's nucleus, central tegmental tract, medial lemniscus, medial longitudinal fasciculus, superior cerebellar peduncle, posterior column, and spinocerebellar tract were all degenerated. Argyrophilic and basophilic neuronal or glial cytoplasmic inclusions immunoreactive for FUS, GRP78/BiP, p62 and ubiquitin were detected in affected lesions. FUS-positive neuronal and glial inclusions were more frequently and widely observed than basophilic, ubiquitin-, and silver-positive inclusions.

Discussion and conclusions: The *FUS* R521C mutation in this Japanese family caused familial ALS with pathological features of multiple system degeneration and neuronal basophilic inclusion.

P104 NOVEL MISSENSE AND TRUNCATING MUTATIONS IN FUS/TLS IN FAMILIAL ALS

WAIBEL S[1], NEUMANN M[2], RABE M[1], MEYER T[3], LUDOLPH AC[1]

[1]*Department of Neurology, University of Ulm, Ulm, Germany,* [2]*Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland,* [3]*Department of Neurology, Humboldt University Berlin, Berlin, Germany*

E-mail address for correspondence: stefan.waibel@uni-ulm.de

Keywords: FUS/TLS, genetics

Background: Mutations in the *FUS/TLS* gene have been associated with familial amyotrophic lateral sclerosis (fALS).

Methods: We analyzed the presence and frequency of *FUS/TLS* mutations in a German ALS cohort, including 133 sALS and 58 fALS patients by sequence analysis of exons 13–15.

Results: We identified two novel heterozygous FUS/TLS mutations in four German ALS families including the missense mutation K510R and the truncating mutation R495X. The truncating mutation was associated with an aggressive disease whereas the K510R variant showed a mild phenotype with a life expectancy of more than 8 years which was seen in each patient, including monozygotic twins. No mutation was detected in 133 sALS patients.

Conclusions: Mutations in FUS/TLS account for 7% (4 of 58) of fALS in our German cohort. In comparison to the frequency of SOD1 mutations these FUS/TLS variants are comparatively rare in our patients.

P105 IDENTIFICATION OF A FUS/TLS GENE MUTATION IN A COHORT OF CATALAN FALS PATIENTS

GAMEZ J[1,2], SYRIANI E[1,3], MORALES M[1,3]

[1]*ALS Unit, Neurology Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain,* [2]*Autonomous University of Barcelona, Barcelona, Spain,* [3]*University of Barcelona, Barcelona, Spain*

E-mail address for correspondence: 12784jgc@comb.es

Keywords: fused in sarcoma (FUS/TLS), p.R521C, TARDBP

Background: A new causal gene for ALS was discovered in early 2009, when mutations in the *fused in sarcoma/translated in liposarcoma (FUS/TLS)* gene were found to segregate with ALS in families linked to chromosome 16. Two articles described 15 *FUS/TLS* mutations in 26 unrelated ALS pedigrees. Subsequent studies in other populations identified *FUS/TLS* mutants in familial ALS, some sporadic ALS cases and in one patient with frontotemporal lobar degeneration (FTLD). The emergence of these results in such a short timeframe suggests that mutations in *FUS/TLS* represent the second most common genetic cause of FALS (the first being *SOD1* mutations). FALS linked to the *FUS/TLS* gene

is designated as ALS6 (MIM 608030). There appears to be an allelic heterogeneity in ALS6, as at least a hundred sequence variants have been described to date, and pathogenicity has been described in 38 of these. Clinical-genetic characterisation of ALS6 is therefore essential for providing information on the phenotype associated with a given mutation, the distributions of *FUS*/*TLS* mutations in different ethnic groups and clarification of the genotype-phenotype correlation in patients with *FUS*/*TLS* gene mutations. No *FUS*/*TLS* mutations have been reported in the Spanish population to date.

Objectives: To investigate the prevalence of *FUS*/*TLS* mutations in a Catalan cohort of patients with familial ALS, in which we carried out a mutational study of *SOD1* in 2006 (1).

Materials and methods: Twenty-five of the families not carrying *SOD1* mutations were screened for *FUS*/*TLS* mutations using direct sequence. Mutations in the *TARDBP* genes were excluded beforehand.

Results: One of the 25 FALS pedigrees (4%) carried a C-to-T transition at nucleotide position 1561 (c.1561C>T) leading to a p.R521C sequence change at protein level. The phenotype in this family was characterized by a relatively young main age at onset (36.8 years old), predominantly lower motor neuron signs and variable survival.

Conclusions: This is the first ALS6 mutation identified in Spain. The prevalence of *FUS*/*TLS* mutations is similar to that reported in other countries. Our findings suggest that *FUS*/*TLS* mutations are the second most common cause of FALS in our population. The fact that *p*.R521C is the most common mutation described in patients from different ethnic backgrounds means that future research should focus on a worldwide haplotype study of pedigrees carrying p.R521C in order to investigate the possibility of a common founder.

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P106 IDENTIFICATION OF NOVEL FUS MUTATIONS IN SPORADIC AND FAMILIAL ALS CASES

BELZIL V[1], DAOUD H[1], ST-ONGE J[1], DESJARLAIS A[1], BOUCHARD J-P[2], DUPRÉ N[2], CAMU W[3], DION P[1], ROULEAU G[1]

[1]*Centre for Excellence in Neuromics, University of Montreal, the Centre Hospitalier de l'Université de Montréal (CHUM) and Ste-Justine Hospital, Montreal, Quebec, Canada,* [2]*Faculty of Medicine, Laval University, Centre Hospitalier Affilié Universitaire de Québec – Enfant-Jésus Hospital, Quebec City, Quebec, Canada,* [3]*Unité de Neurologie Comportementale et Dégénérative, Institute of Biology, Montpellier, France*

E-mail address for correspondence: verobelzil@hotmail.com

Keywords: FUS gene, splicing mutation

Background: Amyotrophic lateral sclerosis is the most common of the motor neuron diseases and is characterized by the degeneration of upper and lower motor neurons localized in the motor cortex, brain stem and spinal cord. So far, many mutations have been identified in *SOD1*, *TARDBP*, and more recently in *FUS*, all of which contribute to a better understanding of the pathological mechanisms involved in ALS. Specifically, mutations identified in *FUS* have been mostly found in FALS cases, specifically in the carboxy terminal of the protein.

Methods: Following the identification of several *FUS* mutations in FALS and a few in SALS, we wanted to evaluate to proportion of *FUS* mutations in the FALS and SALS populations. Therefore, the 15 exons of the *FUS* gene were sequenced in a cohort of 475 French and French-Canadian SALS patients, 475 matched controls, and 156 FALS of mixed origin.

Results: Two missenses, two deletions, one frameshift, one splice mutation and one nonsense mutation in a total of 8 FALS/SALS patients were identified. Among those, one deletion (G175del) was found in a FALS patient, like previously reported, and one missense was reported to be found in one control (G226S). The five other variants, P18S, G144_Y149del, R503fs, R514_Y526del and Q519X were new. The last three variants were located in the carboxy terminal of the protein where the previously reported variants were mostly clustered. One of those is a splicing mutation that is shared by all nine affected members of a large Spanish family. In addition, one new missense (R383C) and one mutation previously identified in a FALS patient (R216C) were found in two control participants. Synonymous variants were found in five SALS and four control individuals.

Conclusion: Our study identified new mutations in SALS patients as well as a new splicing mutation in an ALS family from Spain. This finding will help to better understand the contribution of *FUS* mutations in the ALS pathology.

P107 *DE NOVO* TRUNCATING FUS GENE MUTATION AS A CAUSE OF SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

DEJESUS-HERNANDEZ M[1], KOCERHA J[1], FINCH N[1], CROOK R[1], BAKER M[1], DESARO P[2], JOHNSTON A[2], RUTHERFORD N[1], WOJTAS A[1], KENNELLY K[2], WSZOLEK Z[2], GRAFF-RADFORD N[2], BOYLAN K[2], RADEMAKERS R[1]

[1]*Department of Neuroscience,* [2]*Department of Neurology; Mayo Clinic, Jacksonville, Florida, United States*

E-mail address for correspondence: dejesushernandez.mariely@mayo.edu

Keywords: FUS, de novo, mutation

Background: Mutations in the gene encoding fused in sarcoma (*FUS*) were recently identified as a novel cause of amyotrophic lateral sclerosis (ALS), emphasizing the genetic heterogeneity of ALS and providing novel insights into its pathogenesis.

Objective: To determine the frequency and spectrum of mutations in *FUS* in a consecutive clinical cohort of ALS patients ascertained at Mayo Clinic Florida.

Methods: We sequenced the superoxide dismutase (*SOD1*), TAR DNA-binding protein 43 (*TARDBP*) and *FUS* genes in 99 sporadic and 17 familial ALS patients ascertained at Mayo Clinic Florida. In one family, segregation analysis was performed to determine the origin of the *FUS* mutation. *FUS* transcript analysis was performed in two patients carrying novel *FUS* mutations. Subcellular localization of recombinant FUS proteins was studied in N2A cells using immunocytochemistry and immunoblot analysis.

Results: We identified two novel mutations in *FUS* in two out of 99 (2.0%) sporadic ALS patients and established the *de novo* occurrence of one *FUS* mutation. In familial patients, we

identified three (17.6%) *SOD1* mutations, while *FUS* and *TARDBP* mutations were excluded. The *de novo FUS* mutation (g.10747A>G; IVS13-2A>G) affects the splice-acceptor site of *FUS* intron 13 and was shown to induce skipping of *FUS* exon 14 leading to the C-terminal truncation of FUS (p.G466VfsX14). Subcellular localization studies showed a dramatic increase in the cytoplasmic localization of FUS and a reduction of normal nuclear expression in cells transfected with truncated compared to wild-type FUS. We further identified a novel in-frame insertion/deletion mutation in *FUS* exon 12 (p.S402_P411delinsGGGG) which is predicted to expand a conserved poly-glycine motif.

Discussion: We extend the mutation spectrum in *FUS* leading to ALS and describe the first *de novo* mutation in *FUS*.

P108 PRIORITIZATION OF ALS CANDIDATE GENES THROUGH INTEGRATIVE PATHWAY ANALYSIS

KENNA K[1], HARDIMAN O[1,2], BRADLEY D[1]

[1]*Trinity College Dublin, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland*

E-mail address for correspondence: kennak@tcd.ie

Keywords: candidate, sequencing, pathway

Background: Mutations in 8 genes have been demonstrated to co-segregate with familial ALS. Such variants cause disease through their influence on a single or multiple biological processes. Variation in other genes capable of influencing the same biological processes may also cause disease. For example genetic variation in APP, PSEN1 or PSEN2, can cause familial forms of Alzheimer's disease.
Continual advances in DNA sequencing based technologies provide the opportunity for significantly larger scale interrogation of the genomes of ALS patients for disease relevant variation. Our work is centred on elucidating the genetic basis for ALS in the Irish population by screening candidate genes selected primarily by; 1) position of their encoded proteins within the human protein interaction network relative to proteins encoded by known ALS genes, and 2) similarity of their proteins to ALS proteins in terms of sequence and annotation.

Methods: Candidate Selection: Experimentally verified human protein interactions were retrieved from online database and supplemented with interactions manually curated from the literature to create a model of the human protein interactome. First order interaction partners of known ALS proteins and the second order interaction partners achieving the best prioritization scores were selected as candidates. Predicted paralogs of ALS proteins were also included with second order interactors for prioritization.
Prioritization technique: An in-house version of Google's PageRank algorithm was written and used to score candidates based on position within our model of the human protein interactome relative to that of known ALS proteins. The online tool ToppGene was used to score candidates based on similarity to ALS proteins in terms of numerous annotation features based on protein function, localization and expression. Candidates were also scored by sequence similarity to known ALS proteins using BLASTp. Scores were integrated to an overall score. A weighted profile of genes potentially relevant to ALS was constructed based on various lines of evidence such as occurrence within a known ALS linkage region etc and used to adjust final prioritization.

Results: 283 first and 4140 second order interaction partners of ALS proteins were identified; 321 predicted paralogs were recovered based on an e-value threshold of 10; The coordinates of the exons of 600 genes have been submitted to Agilent for RNA capture probe design to allow for subsequent sequencing.

Conclusion: Current technologies allow much higher throughput screening of candidate genes for disease relevant variation. Genes capable of influencing similar biological processes to known ALS genes are worthy candidates, and we have integrated multiple approaches to prioritize the most plausible of such candidates for sequencing in ALS patients.

P109 IMMUNOLOGIC INVESTIGATIONS IN AMYOTROPHIC LATERAL SCLEROSIS: CYTOKINE POLYMORPHISMS AND RELATED MRNA LEVELS IN LYMPHOCYTES OF SALS PATIENTS

GAGLIARDI S[1], BOIOCCHI C[2], COVA E[1], MILANI P[1], ALVISI E[3], CERONI M[1,3], CUCCIA M[1], CEREDA C[1]

[1]*Laboratory of Experimental Neurobiology, IRCCS, Neurological Institute "C. Mondino", Pavia, Italy,* [2]*Department of Genetics and Microbiology, University of Pavia, Pavia, Italy,* [3]*Division of General Neurology IRCCS, Neurological Institute "C. Mondino", Pavia, Italy*

E-mail address for correspondence: cristina.cereda@mondino.it

Keywords: cytokines, polymorphisms

Background: The implication of the immune system in ALS is supported by data on levels of cytokines that have been found increased in serum and in cerebrospinal fluid of ALS patients (1). Interleukin 7, 9, 12, 17 and IL-1beta (IL-1ß) levels were found higher in CSF (2) and Antigenic Tumor Necrosis Factor-α (TNF-α) have been found increased in sera from ALS patients (3).

Objectives: This study analyzed 23 genetic polymorphisms of the 13 cytokines genes and we evaluated lymphocyte mRNA levels of the genes that showed statistically significant differences in alleles and genotype frequencies between ALS patients and controls.

Methods: Allelic, genotypic and haplotype frequencies of the polymorphisms in IL-1α, IL-1β, IL1-R, IL1-RA, IL-4Rα, IL-12, IFN-g, TGF-β, TNF-α, IL-2, IL-4, IL-6, IL-10 genes were assessed in 60 ALS patients and 140 healthy controls by RFLP. A Real-Time Sybr Green qPCR was used for TNF-α, IL-1β and TGF-1β expression analysis in 35 SALS patients and 35 controls. Normalization was optimized using YWHAZ as a housekeeping gene.

Results: Our data showed statistically significant differences in CC/CT genotype distribution of IL-1β (P= 0.0347) and CG/GG genotypes of TGF-1β (P=0.013). Moreover, there was a decrease in CT genotype frequencies in the IL-1β gene (P=0.0149) and CG genotype in the TGF-1β gene (P=0.0126) in ALS patients compared to controls. Our data showed a significant increase of AA genotype (P=0.019) for -238 TNF-a polymorphism in patients compared to controls. Our study shows that TNF-α and IL-ß1mRNA levels from SALS lymphocytes were expressed at higher level than in controls (P<0.05). TGFß1 mRNA quantity was higher in SALS samples than in control, although this did not reach statistical significance. Gene polymorphisms were not associated with clinical features and mRNA level.

Discussion: Our data suggest a common function of TNF-α and IL-1ß in ALS. Increased TNF-α and IL-1ß gene expression may be due to neuroprotective properties. TNF-α could be a response to ROS increase, documented in ALS disease.

Hydrogen peroxide is well known as an activator of TNF-α gene expression through NF-kb (3). *In vitro* experiments have demonstrated that TNF-α down-regulates hSOD1 promoter via JNK/AP-1 signalling pathway. The increase of TNF-α demonstrated in this work, might be one of the causes of reduced levels in lymphocytes of SOD1 observed in patients with sporadic ALS (4). As TNF-α, IL-1ß has a neuroprotective role by inducing NGF production by enhancing ROS level. We plan to deeply investigate the immunogenetics of ALS disease in order to identify the cytokines' mechanisms in the development of this complex multifactorial disease.

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P110 INVESTIGATING THE GENETIC BASIS OF ALS AND OTHER MOTOR NEURON DISEASES: ANALYSIS OF KNOWN GENES AND SEARCH FOR NEW LOCI

WILLIAMS K[1,2], DREW A[1,2], SOLSKI J[1], DURNALL J[1], THOENG A[1,2], WARRAICH S[1,2], THOMAS V[1,2], CRAWFORD J[1,3], NICHOLSON G[1,4], BLAIR I[1,2]

[1]*ANZAC Research Institute, Sydney, NSW, Australia,* [2]*University of Sydney, Sydney, NSW, Australia,* [3]*Macquarie University, Sydney, NSW, Australia,* [4]*Concord Hospital, Sydney, NSW, Australia*

E-mail address for correspondence: iblair@med.usyd.edu.au

Keywords: gene, linkage, next-generation sequencing

Background: The motor neuron disorders are a group of neurodegenerative diseases that cause the selective progressive death of motor neurons. MND ranges from the rapidly progressive fatal form, amyotrophic lateral sclerosis (ALS), to slowly progressive forms including the lower motor neuron disorder, hereditary motor neuropathy (HMN). Genetic and clinical overlaps between fatal and non-fatal MNDs imply shared pathogenic pathways. Familial ALS (FALS) accounts for approximately 10% of ALS cases with the remainder being sporadic (SALS). MND is genetically heterogeneous. To date, known genes account for a small proportion of cases.

Objectives: We aim to investigate known ALS/MND genes and identify new ALS/MND genes in large cohorts of Australian ALS and non-fatal MND families.

Methods: Large cohorts of Australian ALS families (n=147) and families with non-fatal MND (n=24) have been recruited. We are using a combination of traditional genetic linkage approaches together with next-generation sequencing strategies to search for new ALS/MND genes among families that are negative for all known ALS and other MND genes. Known ALS/MND genes have been the subject of mutation analysis among these cohorts.

Result: We analysed 147 ALS families for mutations in *SOD1*, *DCTN1*, *ANG*, *CHMP2B*, *VEGF*, *FIG4*, *TARDBP*, *FUS*, and *DAO*. Haplotype analysis was also performed to identify any potential founder mutations. We determined that mutations in known ALS genes account for 20% of Australian ALS families, and comprises of *SOD1* (14.3%), *FUS* (2.7%), *ANG* (2.0%), and *TARDBP* (1.4%) mutations. None of the identified mutations in *SOD1* and *ANG* were present in a screen of 492 control chromosomes. Among families with identified mutations, variable phenotypic expressivity was observed with gene and mutation specific effects for disease onset and duration. To identify new loci for familial ALS and non-fatal MND, genome-wide linkage scans were performed in a subset of families that were negative for all known genes. An 8 cM genome-wide microsatellite scan was carried out by deCODE-Iceland using 166 individuals (affected, unaffected and obligate carriers). Subsequent analyses have yielded significant and suggestive linkage to several chromosomal regions. Families are undergoing fine genetic mapping using additional genetic markers to assist in refining loci and excluding false-positive regions. Target-region sequencing and exome capture-sequencing (NimbleGen capture-Solexa sequencing) have also been performed on selected families with data analysis underway.

Discussion and conclusions: The genetic defects are yet to be identified among 80% of ALS families (117/147 families) within our cohort. The chromosomal regions implicated from our genome-wide linkage scans do not overlap previously identified loci, implicating substantial genetic heterogeneity. Identification of novel ALS/MND genes will give insights into the biological basis of both familial and sporadic motor neuron degeneration, allow development of new disease models and provide new targets for therapeutic development.

P111 USING EXOME SEQUENCING TO IDENTIFY GENES CAUSATIVE FOR FAMILIAL ALS

BROWN, JR RH[1], TICOZZI N[2], GELLERA C[3], LECLERC AL[1], KEAGLE P[1], MCKENNA-YASEK DM[1], SAPP PC[1], GLASS JD[4], SILANI V[1], LANDERS JE[1]

[1]*Department of Neurology, University of Massachusetts Medical School, Worcester.MA, United States,* [2]*Department of Neurology and Laboratory of Neuroscience, "Dino Ferrari" Center, Università degli Studi di Milano- IRCCS Istituto Auxologico Italiano, Milan, Italy,* [3]*Units of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy,* [4]*Department of Neurology, Emory University, Atlanta, GA, United States*

E-mail address for correspondence: john.landers@umassmed.edu

Keywords: next-generation sequencing, exome sequencing, gene identification

ALS is an adult-onset, rapidly progressive, and ultimately fatal neurodegenerative disease caused by the selective loss of motor neurons. To date, the underlying cause of familial ALS (FALS) has been identified only in ~35% of all FALS cases. Approximately 20% of FALS cases are caused by mutations in SOD1, ~5% in TARDBP and ~5% in FUS; mutations in several other genes have been found in isolated families. Genetic studies have provided invaluable information towards the understanding of pathogenesis in both FALS and SALS. Undoubtedly, the discovery of novel FALS-associated genes will dramatically further our knowledge of the cellular pathways that lead to motor neuron degeneration.

To date, two strategies typically have been used to identify new genes in monogenic diseases: linkage analysis and candidate gene analysis. Linkage analysis requires large pedigrees composed of many affected individuals in different generations; this task is considerably difficult in ALS, a disease of adult life with a rapid disease course. On the other hand, candidate gene analysis, while feasible in cohorts of unrelated FALS cases, is flawed by a selection bias. Until now, it was not economically feasible to screen for rare variants at a genome-wide scale on large cohorts. However, the recent advances in automated short-read DNA sequencing offer new solutions to this problem. It is now possible to sequence only protein-coding regions of the genome (exomes) to reduce costs while enriching for discovery of a highly penetrant variant. Towards this end, we have embarked on an ongoing project to identify novel FALS variants using exome sequencing. In this presentation, the methodology of exome

sequencing will be illustrated as well as the pitfalls of this approach. Recent results from ongoing studies in FALS families analyzed using exome capture and next-generation sequencing will be discussed.

P112 GENE SET ENRICHMENT ANALYSIS OF GENE EXPRESSION PROFILES OF LYMPHOCYTES FROM AMYOTROPHIC LATERAL SCLEROSIS PATIENTS PREDICTS DEREGULATION OF THE UBIQUITIN/ PROTEASOME PATHWAY

MOUGEOT J-L[1,2], PRICE A[1], ARMSTRONG E[1], BROOKS BR[1]

[1]*Carolinas Medical Center, Department of Neurology, Carolinas Neuromuscular/ALS-MDA Center, Charlotte, NC, United States,* [2]*The University of North Carolina at Charlotte, Charlotte, NC, United States*

E-mail address for correspondence: jean-luc.mougeot@carolinas.org

Keywords: microarray, GSEA, ubiquitination

Background: Recently, Saris *et al.* (2009) have found neurological disease-related molecular signatures in total blood from ALS patients compared to healthy controls using weighted gene co-expression network analysis. We applied gene set enrichment analysis (GSEA; BROAD Institute, Cambridge, MA) to a microarray dataset generated in our laboratory from purified lymphocytes from ALS patients and healthy controls.

Objectives: To analyze an existing (unpublished) microarray dataset produced in our laboratory using GSEA and to confirm predicted pathway alteration in peripheral blood mononuclear cells (PBMCs) at the protein level by Western blot analysis.

Methods: GSEA was applied on a microarray dataset obtained from lymphocytes isolated from definite sporadic ALS patients (ALS, n=11) and healthy controls (HC, n=11) using Agilent 4X44K Whole Human Genome Microarrays in a dual-mode reference design. The MIDAS/TM4 program was used for LocFit-LOWESS normalization and to generate two filtered datasets, DS3500 (10,147 probes) and DS7000 (7,199 probes), where both Cy3 and Cy5 integrated intensities were above one or two standard deviation(s) of their respective background. Log_2 of ratio of classes and t-test were used as ranking metrics. Datasets were computed against generic human gene sets of the GSEA/MSigDB v2.0.5 database. GSEA analysis was also performed using keywords such as aging, mitochondrion, SOD1, proteasome and ubiquitin, to extract gene sets from MSigDB. Western blot analysis was used to determine total ubiquitination in PBMCs from additional ALS patients (n=10), HC (n=10) and patients with multiple sclerosis (MS, n=10).

Results: A total of 130 gene sets among 4995 were found significant ($P<0.05$). Chromosomal location gene set CHR5Q14 contained COX7C which polymorphisms may be associated with ALS. Four gene sets were significant in all comparisons ALS vs. HC: HSA04120_UBIQUITIN_MEDIATED_ PROTEOLYSIS, CANCER_NEOPLASTIC_META_UP, CALRES_RHESUS_DN and NOUZOVA_ CPG_H4_UP. Most enriched gene sets were associated with DNA repair, histone acetylation and ubiquitin-mediated proteosomal degradation. Mitochondria appeared marginally affected as lumen and matrix related gene sets were only significant using Log_2 of ratio of classes as a metric. GSEA based on keywords (see Methods) also suggested alteration of ubiquitination/proteasome function. Western blot analysis of PBMCs from additional ALS and MS patients and HC subjects confirmed increased levels of ubiquitinated proteins in ALS as well as in MS patients.

Discussion and conclusions: GSEA was found to be a powerful method to reveal unifying biological themes regarding our ALS microarray dataset, as relevant biological differences are modest relative to the noise at the genome-wide scale. GSEA was predictive of changes in ubiquitination in PBMCs from ALS patients also occurring in MS patients. Thus, in the search for biomarkers that relate to ALS etiology, it is critical to include relevant disease controls in microarray studies.

P113 TOWARD DISCOVERY OF ALS-SPECIFIC GENE NETWORKS BY AUTOMATIC LITERATURE ANALYSIS

BUCCI EM[1], POLI A[1], NATALE M[1], BONINO D[1], MARCHIARO M[1], GULLUSCI M[1], MONTAGNOLI L[1], CONSOLI P[1], VINCI E[1], ABRESCIA C[2], BONGIOANNI P[3,2]

[1]*Biodigitalvalley Srl, Pont Saint Martin (AO), Italy,* [2]*NeuroCare onlus, Pisa (PI), Italy,* [3]*Azienda Ospedaliero-Universitaria Pisana, Pisa (PI), Italy*

E-mail address for correspondence: paolo.bongioanni@tin.it

Keywords: bioinformatics, gene networks

Background: In former years, attention has been paid to the system biology of Amyotrophic Lateral Sclerosis (ALS), ie to the identification of relationships between different players in the cascade of molecular events regulating ALS. In particular, methods were developed to automatically parse the scientific literature, to identify co-occurring names of proteins/genes and to identify terms which qualify the relationships between them. However, since most of the available methods parse only scientific abstracts, the information obtained is often incomplete, due to the fact that only those proteins which are in the main scope of the paper are discussed, while often data on a number of other proteins are contained elsewhere in the relevant papers.

Objectives: To overcome these limitations, we focussed on the analysis of the figure captions contained in the scientific literature. We apply this method to obtain a robust biological network of genes involved in ALS.

Methods: The captions of a paper often contain an enriched amount of data on different proteins and on their interconnections, ie terms referring to proteins and simultaneously those referring to experimental methodologies. Analyzing captions allows identification of groups of proteins studied with a certain experimental technique, while at the same time characterizing the relationships among them. For example, proteins co-occurring in a caption describing a double-hybrid experiment are most likely binding partners, while proteins co-occurring in a caption describing a 2D-gel experiment are probably co-expressed in a given condition.
In our study, more than 2,000,000 papers were examined, using ad-hoc JAVA codes for parsing the pdf documents, identifying captions, and matching relevant terms. Identified genes were classified on the basis of their involvement in ALS only, in ALS and other neurodegenerative conditions, or in ALS and other non-neurodegenerative conditions.

Results: We found that: 1) by our method, some genes could be linked to ALS on the basis of publications preceding the demonstration of their direct involvement in ALS; 2) the obtained network matches quite well to the corresponding curated public-domain databases. Moreover, of relevance to ALS molecular biology, we found that: 3) new genes are

identified, thus suggesting potential new targets for experimental studies; 4) some genes appear specifically connected to ALS, potentially differentiating this neurodegenerative condition from others.

Discussion and conclusions: Unravelling the intricacy of protein relationships defining the system biology of ALS involves building robust networks of protein biological interactions, including both genes already related to ALS and without recognized clinical relevance, but having biological meaning.
We found that supplementing usual methods of automatic literature analysis, with the analysis of the experimental data contained in captions, can produce better results, and leads to the identification of new potential targets for the molecular characterization of ALS.

P114 THE ALS ONLINE GENETICS DATABASE, ALSOD

ABEL O, POWELL J, ANDERSEN P, AL-CHALABI A

Department of Clinical Neuroscience, Institute of Psychiatry, King's College London, London, United Kingdom

E-mail address for correspondence: abstracts@mndassociation.org

Keywords: genome wide study, database, bioinformatics

Background: The ALS Online Genetics Database, ALSOD (http://alsod.iop.kcl.ac.uk/), is a central repository for genetic information on ALS. The initial aim of this database was to discover genotype: phenotype (G2P) correlations for SOD1 mutations. There has been considerable progress both technologically and scientifically since ALSOD was originally setup in 1999, presenting an opportunity for the collection and presentation of ALS genetic data in a single repository. The database is being transformed to meet these challenges.

Objectives: To provide a single, continuously updated central repository summarizing the current state of ALS genetics using open source standards and providing a service to the ALS research community, including automated meta analysis and integration of data from linkage and association studies.

Methods: The requirements of the ALS genetics research community were collected from the ALSOD feedback page. The database schema was restructured to allow for flexibility and expansion by changing table designs, rewriting queries and implementing appropriate stored procedures. Codes and scripts were written in programming languages like javascript, XML, C#, T-SQL and VB.NET integrated under the ASP.NET platform.

Results: The user registration process and data submission methods have been simplified and streamlined. ALSOD has been accessed over 141,000 times since January 2009 by users from 124 countries, with 11,604 accesses in the month of May alone. An overview of key published studies for approximately 43 ALS-related genes (http://alsod.iop.kcl.ac.uk/overview/index.aspx) is now included. Bioinformatics and analysis tools such as PLINK and Haploview have been integrated into the web-pages. Existing genome-wide association study data have been collected for meta-analysis and on-the-fly meta-analysis in which unpublished user-data is combined with existing studies confidentially and the result fed back in minutes. Links to gene variants, gene databases and relevant publications have been manually added to web-pages, but this process will be automated. Google AJAX search and Google Earth API have been combined to overlay geographical SOD1 mutations on the globe. This will be extended to other genes.

Discussion and conclusions: Advances in genetics and the fast pace of publications mean that a central resource summarizing all available data in an easily digestible form is essential. Integration with existing databases and analysis software means that ALSOD is a powerful resource for exploring existing genetic information for ALS. New tools are in development and these will be made live after initial beta-testing is passed. We welcome ideas for development or the addition of open source code from the user community. ALSOD is widely used by the ALS genetics research community. We aim to make it an indispensable tool for ALS research.

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THEME 6 EPIDEMIOLOGY

P115 IMPLEMENTATION OF A NATIONAL AMYOTROPHIC LATERAL SCLEROSIS REGISTRY

HORTON KD, MURAVOV O, KAYE W, SANCHEZ M

ATSDR/DHS/SRB, Atlanta, GA, United States

E-mail address for correspondence: ffw2@cdc.gov

Keywords: registry, national databases, pilot projects

The uncertainty about the incidence and prevalence of Amyotrophic Lateral Sclerosis (ALS), as well as the lack of knowledge about the role of environmental exposures in the etiology of ALS, have created a need for structured data collection through a national ALS registry. In 2008, President Bush signed a law that provides for a national ALS registry to be created. Prior to this law being enacted, the Agency for Toxic Substances and Disease Registry (ATSDR) was already conducting four pilot projects (during 2006–2009) to determine the feasibility of creating a national ALS registry. Results from the pilot projects concluded that a national ALS registry is feasible; however, several different methodologies would be needed for identifying a large portion of individuals with ALS. Therefore, in 2009, ATSDR began implementation of the National ALS Registry using a two-pronged approach to help identify all US cases of ALS. The first approach, currently being undertaken, utilizes existing national administrative databases to identify prevalent cases, based on an algorithm developed from the pilot projects. Results from the national administrative databases will be presented if available. The second approach, which will be implemented in fall 2010, will use a secure web portal to identify cases missed by the national administrative databases. Also, to improve the completeness of the National ALS Registry, ATSDR is concurrently implementing registries that will allow for timely population-based case estimates of ALS in smaller defined geographic areas (ie, at the state and metropolitan levels). The purpose of this presentation is to provide an overview of the development and implementation of the National ALS Registry.

P116 MULTI-CENTER, INTERNET-ACCESSIBLE ALS PATIENT CLINICAL OUTCOMES REGISTRY CONTAINING DE-IDENTIFIED CLINICAL DATA DRAWN FROM THREE US CENTERS

BOYLAN K[1], BROOKS BR[2], GRAVES M[3], SEPULVEDA L[3]

[1]*Mayo Clinic, Jacksonville, Florida, United States,* [2]*Carolinas Medical Center, Charlotte, North Carolina, United States,* [3]*University of California, Los Angeles, California, United States*

E-mail address for correspondence: boylan.kevin@mayo.edu

Keywords: outcomes registry, database, software

Background: Patient registries are integral to clarifying the clinical course, prognosis and treatment of ALS. Progress in this area has been limited by lack of standardized software tools that are readily accessible, clinically integrated, searchable, and analytically practical at both the individual patient and aggregate level across multiple centers.

Objectives: Demonstrate feasibility of a standardized, internet-accessible archive of pooled, de-identified clinical data on ALS patients from site specific databases at multiple centers to serve as a basis for multi-center clinical and epidemiological research in ALS.

Methods: Using a commercially available program, the University of California, Los Angeles (UCLA), Carolinas Medical Center and Mayo Clinic Florida (MCF) established a common data set based on core data elements used in the NINDS DNA banking repository (1) to create an independent registry of de-identified clinical data. The 1275 page Data Dictionary was reiteratively tested and modified at three separate sites to provide minimal and expanded data element capability. Each center also customized the database registry based on individualized requirements at the respective centers, including server hosting.

Results: The ALS database at UCLA functions as an electronic medical record containing medical records, clinical and laboratory data for the ALS clinic, supports generation of prescriptions for equipment and medications, and allows patients and caregivers to enter data online and receive educational materials. The databases at all 3 centers serve as patient registries to support research. The MCF database also supports data collection in ALS clinical trials. The database program allows export of de-identified batch data and uploads into a central, multi-center registry (about a 10 minute process). The multi-center database, maintained on a secure server, is not directly linked to the database at any of the 3 centers.

Conclusion: We demonstrate feasibility of an internet-accessible, multi-center ALS patient registry containing de-identified common data elements uploaded from actively maintained databases at 3 independent ALS centers, driven by off-the-shelf commercially available database software.

Discussion: A common database program with shared field codes facilitates data sharing without compatibility issues or need for data transformation. Data transfer to the multi-center registry is controlled by source data administrators at each site, without risk of external, unauthorized access to the source database. The source databases cannot be accessed by users of the multi-center database. The database program supports longitudinal data collection, clinical trial management, and a patient portal for data entry and delivery of educational materials. The Data Dictionary will be made freely available to all ALS Clinic sites that want to enter into a national ALS patient clinical outcomes registry that might be a means to permit Joint Commission Accreditation Program ALS Disease Specific Certification or patient safety registries.

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P117 A POPULATION-BASED STATEWIDE REGISTRY OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS IN MASSACHUSETTS

KNORR R[1], CONDON S[1], FISCHETTI C[1], ARMON C[2]

[1]*Massachusetts Department of Public Health, Boston, MA, United States,* [2]*Baystate Medical Center, Springfield, MA, United States*

E-mail address for correspondence: christine.fischetti@state.ma.us

Keywords: registry, epidemiology, prevalence

Background: Prior to the development of the Massachusetts ALS Registry, there have been no population-based registries for ALS in the US. Beginning in 2003, following a legislative mandate and the inclusion in state regulations of ALS as a reportable condition possibly linked to environmental exposures, Massachusetts has been developing the tools needed to establish a statewide registry. Following completion of pilot studies and focus groups, registry data collection was initiated on January 1, 2008 for the ascertainment of 2007 prevalent cases.

Objectives: The primary objective of the Registry is to identify all prevalent and incident cases of ALS in Massachusetts in order to better understand trends and patterns of the disease. The secondary objective is to create a patient database to stimulate research into causes and/or treatments of ALS.

Methods: Primary data sources are 650 neurologists in private, clinic, or hospital practice and the medical record department of hospitals. Secondary data sources, such as death certificates, hospice organizations, and patient advocacy groups are used to determine if cases are missed using primary sources. For each patient identified, trained nurses abstract a standardized set of data at each practice and photocopy the source documentation. The diagnosis of ALS is classified according to the revised World Federation of Neurology criteria for the diagnosis of ALS by neurologists experienced in applying these criteria.

Results: The collection of 2007 prevalent cases has been completed and data collection is on-going for subsequent years. The crude statewide prevalence was 4.62/100,000, which includes Definite, Probable, and Lab-supported Probable ALS based on El Escorial criteria. Upon inclusion of Possible ALS, the crude prevalence was 4.97/100,000. Inclusion of Primary Muscular Atrophy and Primary Lateral Sclerosis cases, which make up a portion of the Suspected ALS category, lead to a crude prevalence of 5.96/100,000. The breakdown of eligible cases by El Escorial criteria is as follows: 18.0% definite ALS; 20.4% probable ALS; 13.1% laboratory-supported probable ALS; 4.0% possible ALS; 19.7% suspected ALS. 19.7% of patients did not have ALS according to the El Escorial criteria. The remaining cases were unable to be analyzed due to insufficient information from medical records.

Discussion and conclusions: Results will be analyzed by community in Massachusetts to evaluate trends and patterns throughout the state. As additional years of data are added to the registry, we will have greater statistical power to investigate etiologic hypotheses such as the role the environment may play in ALS. Our data will also be available to other researchers and public health professionals.

P118 THE LJUBLJANA ALS DATABASE: CLINICAL FEATURES OF OUR PATIENTS

KORITNIK B, ZGONC V, LEONARDIS L, DOLENC GL, RISTIC KS, STUKOVNIK V, ZIDAR I, ZIDAR J

Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia

E-mail address for correspondence: blaz.koritnik@kclj.si

Keywords: database, survival, disease progression

Background: Knowledge of clinical and demographic data on ALS patients is important to identify prognostic factors of the disease. This might help health professionals in better treatment planning and timely introduction of interventions. It can also help patients and their caregivers to better cope with the disease and to schedule their activities during the disease course.

Objectives: The aim of our database is to gather the available clinical and demographic information on the ALS patients seen by the Ljubljana ALS team. We also wanted to use the data to explore the disease course within different subgroups and to look for possible prognostic factors.

Methods: We included all patients with ALS that were referred to be seen by the Ljubljana ALS team between October 2002 and December 2009. The data was gathered at regular patient visits that were scheduled every 3 months. Some of the patient data was collected retrospectively based on patient notes.

Results: 167 (47% men, 53% women) were identified. Mean age at disease onset was 62 years (range 32–82), 63 years in women and 61 years in men. The disease started as spinal form in 66% and as bulbar form in 30%. Four patients (2%) had the familial form of the disease. According to El Escorial diagnostic criteria (EDC) at the time of referral, 29% of patients had definite, 41% probable and 14% possible form of ALS. Walking problems occurred in 88% of patients during the course of the disease. 46% were eventually unable to ambulate independently. 32% percent of patients were unable to feed themselves. 70% had speech problems, 35% became anarthric. Swallowing problems occurred in 72% of patients and in 35% PEG was performed. 64% of patients experienced breathing problems. 23% opted for noninvasive ventilation while only 2% of patients were tracheotomised. The median survival time from symptom onset was 23 months (2–114). The median survival time after diagnosis was 12 months (0–53). In those with PEG, the median survival time after this intervention was 5 months (0–27).

Conclusion: Although not being a population study, by using the database, we were still able to get some interesting insights into the disease course. The database will possibly form the basis for a national ALS registry in Slovenia. This is also a first survey of ALS patients in Slovenia and the data can be used for comparison with other similar studies.

P119 MORTALITY DATA AND DEATH CERTIFICATION FOR AMYOTROPHIC LATERAL SCLEROSIS

GEORGOULOPOULOU E, SOLA P, GOLDONI CA, BERNABEI C, NICHELLI P, MANDRIOLI J,

Department of Neuroscience, Nuovo Ospedale Civile S. Agostino-Estense, University of Modena and Reggio Emilia, Modena, Italy

E-mail address for correspondence: j.mandrioli@ausl.mo.it

Keywords: mortality, epidemiology, death certification

Background: Despite the efforts made to improve the accuracy of death certification for ALS, these data often lack sensitivity and specificity. However, a fairly homogeneous accuracy is reported among different countries: previous studies have found that 72–90% of the cases had ALS listed as the primary cause of mortality on death certification.

Objectives: To evaluate mortality data and the sensitivity and specificity of death certification for ALS in Modena, Italy.

Methods: All the cases deceased during the period 2000–2009 were ascertained through these different sources: 1) the population based registry of ALS in the province of Modena which has been in operation from 2000; 2) the computerized discharge archive of Modena; 3) the death certificates of residents in the province who died from ALS.

Results: From 2000 to 2009 considering all the above-mentioned sources, 142 residents died from ALS; mean age at death was 69 years. Death certificates were available for the totality of the patients. ALS was indicated as the primary cause of death in 119 cases (sensitivity: 83.80%). Mean age at death was 69 years. The number of patients known to have ALS in their lives but not captured by death certificates was 23 (false negatives: 16.20%; mean age at death: 74 years). In these cases cause of death was indicated as pneumonia (22%), heart disease (17%), tumors (14%); stroke, dementia, brain tumor, muscular dystrophy, spine disease were also indicated as primary causes of clinically confirmed ALS cases. In 35% of cases the primary cause was one of the known co-pathologies of the patients. Death occurred at home in the 69% of cases. ALS was listed as a cause of death in 14 cases that were unknown to the registry (11.76%) probably because they were followed in other provinces of Italy. Among these cases, for 9 there were clinical data supporting the diagnosis of ALS and 2 were familial ALS; for 5 cases there was no available record of a diagnosis during life (likely false positive: 4.20%).

Conclusions: These data are quite good if compared with international data on death certificate accuracy, but they underline an evident underreporting of ALS as the primary cause of death. Therefore the use of mortality data based on death certificates as a surrogate of incidence rates is not recommended. Conversely death certification has a low false positive rate and can be useful to integrate clinical data from population based registries, particularly in consideration of the recent introduction of data protection laws.

P120 CHANGES IN THE EPIDEMIOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS IN MODENA, ITALY, FROM 1990 TO 2009

GEORGOULOPOULOU E, SOLA P, BERNABEI C, NICHELLI P, MANDRIOLI J

Department of Neuroscience, Nuovo Ospedale Civile S. Agostino-Estense, University of Modena and Reggio Emilia, Modena, Italy

E-mail address for correspondence: j.mandrioli@ausl.mo.it

Keywords: epidemiology, incidence, prevalence

Background: Few studies deal with the temporal trend of ALS incidence: a study from Rochester, Minnesota, reported a constant ALS incidence rate of 1.7/100,000. Other non population based studies reported increasing ALS incidence rates through the years, and an increasing ALS mortality over time, especially in the elderly. More than one independent study has shown an increasing ALS prevalence and survival in relation to a multidisciplinary approach, to an increased use of non-invasive ventilation and to an increased attention to nutrition.

Objective: To study the changes in ALS epidemiology from 1990 to 2009 in the province of Modena.

Materials and methods: From 1990 through 2009 cases were ascertained from all the neurological centers and hospitals of the province of Modena, death certificates, and the Italian ALS Association. A population based registry of ALS in the province of Modena has been in operation since 2000.

Results: During the period considered, 326 residents (166 men, 160 women) received a diagnosis of ALS. The average annual ALS incidence was 2.58/100,000, with a constant incidence increase with increasing age (mean age at onset: 64 years). Incidence rate was 2.18/100,000 in the years 1990–99 and 2.94/100,000 in the years 2000–09. Mortality rates were quite stable: 1.88/100,000 in the first decade, to 2.17/100,000 in the second decade. The prevalence rates were 1.83/100,000 in 1990, 4.31/100,000 in 2000, and 11.48/100,000 in 2009. The 50% of patients diagnosed after 2000 survived 40 months, whereas the 50% of patients diagnosed before survived 27 months ($P<0.001$ logrank test). Survival was worse in bulbar form and older people; also non-invasive ventilation and PEG use were associated to a longer survival.

Discussion: Comparing the present data with the retrospective ones, we observe a marked increase of ALS prevalence, in spite of a mild increase of incidence and mortality. A more accurate case ascertainment, an improved ALS diagnostic process, and a greater attention to the disease can explain these data. The prevalence values in our province are very high, perhaps in relation to long survivors. The increase in survival may be ascribed to improved patients' care, with a timely treatment of dysphagia, malnutrition, and respiratory failure.

Conclusions: We can conclude that the genuine change in ALS epidemiology over time concerns ALS prevalence and survival. The increase in ALS survival and prevalence, with prevalence rates among the highest previously reported, underlines the importance of palliative care and specialized centers for MND diseases.

P121 A FOLLOW-UP STUDY ON ALS IN THE KOZA/KOZAGAWA/KUSHIMOTO FOCUS AREA OF THE KII PENINSULA FROM THE 1960S TO THE 2000S: A NEW CLUSTER OF ALS

KIHIRA T[1], YOSHIDA S[1], KONDO T[2], OKAMOTO K[3], KOKUBO Y[4], KUZUHARA S[5,4]

[1]*Kansai University of Health Sciences, Kumatori, Osaka, Japan,* [2]*Wakayama Medical University, Kimiidera, Wakayama, Japan,* [3]*Aichi Prefectural University, Nagoya, Aichi, Japan,* [4]*Mie University Graduate School of Medicine, Tu, Mie, Japan,* [5]*Suzuka University of Medical Science, Suzuka, Mie, Japan*

E-mail address for correspondence: tkihira815@kansai.ac.jp

Keywords: Kii-ALS, incidence, calcium

Background: In the 1960s, the incidence of ALS was high in the southern part of the Kii Peninsula, especially in Koza/Kozagawa/Kushimoto area (K area), where the drinking water was supplied from the Kozagawa river and had an extremely low calcium (Ca) content. The incidence of ALS in K area gradually decreased in the 1980s. Recently, however, new patients were found in residents on a small island Oshima close to the K area where no patients had been found between 1965 and 1999. In Oshima, the drinking water previously had contained high Ca until 1975 when the water supply was changed from wells to the Kozagawa river.

Objectives: We investigated the changes in incidence of ALS in K area and Oshima between 1967 and 2008 with special reference to Ca concentrations in drinking water.

Methods: Probable and definite ALS patients, the diagnosis of which was made by neurologists according to the El Escorial criteria in K area and Oshima were collected during three research periods: period I, between 1967 and 1971; period II, between 1989 and 1999; and period III, between 2000 and 2008. The concentrations of Ca and Mg in the drinking water and in the regional river were measured.

Result: The crude incidence of ALS in K area (population, 23,357) was 5.7/100,000 in period III, which was similar to 6.0/100,000 in period I. In Oshima, while no patients were found during periods I and II, the crude incidence was 31.2/100,000 in period III (population, 1,069). The age- and sex-adjusted incidence (in 2000 census) of K area was 2.3/100,000; that of Oshima, 9.0/100,000 and that of Kozagawa, 4.5/100,000 (population, 3,426), in period III. The drinking water of Oshima and Kozagawa contained low Ca (3.0 ppm) similar to that of K area.

Discussion: The recent high crude incidence of ALS in K area might be partly attributable to an increase in the senility rate in the population. In contrast, the age- and sex-adjusted incidence of ALS was high in Oshima and Kozagawa, between 2000 and 2008, although continuous study over a longer period is necessary because of the small population. It is noteworthy that no patient with ALS was found in Oshima for 35 years, in contrast to the K area. The recent low Ca concentration in the drinking water in Oshima might have some role in the appearance of a new high incidence district in the Kii Peninsula.

Conclusions: We have demonstrated that the recent incidence of ALS was high in Kozagawa and Oshima, and that the drinking water of these districts contained low Ca. The relationship between high incidence of ALS and low Ca concentration in the drinking water should be pursued in future.

P122 GENDER DIFFERENCES IN THE RELATIONSHIP BETWEEN LIFESTYLE FACTORS AND RISK OF AMYOTROPHIC LATERAL SCLEROSIS

OKAMOTO K[1], KIHIRA T[2], KOKUBO Y[3], NAGAI M[4]

[1]*Aichi Prefectural University School of Nursing, Nagoya, Japan,* [2]*Kansai University of Health insulance, Osaka, Japan,* [3]*Mie University School of Medicine, Mie, Japan,* [4]*Saitama Medical College School of Medicine, Saitama, Japan*

E-mail address for correspondence: okamoto@nrs.aichi-pu.ac.jp

Keywords: gender, lifestyle factors, epidemiology

Objective: Few human studies have reported sex differences in the relationship between lifestyle factors and the risk of amyotrophic lateral sclerosis (ALS). We therefore analyzed gender differences in the relationship between lifestyle factors and the risk of ALS using a case-control study in Japan.

Methods: The study comprised 183 ALS patients diagnosed by EI Escorial World Federation of Neurology criteria, and 407 gender- and age- matched controls randomly selected from the general population. A structured self-administered questionnaire specifically designed for this case-control study was distributed and collected by mail in both patients and controls. We asked patients to recall their lifestyle within the 3 years before the onset of ALS, and controls within the 3 years before the survey. The strength of association between ALS and a potential risk factor was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs).

Results: An increased risk of ALS was significantly associated with type A behavior pattern and a less frequent intake of green vegetables in both men and women. In males only, vigorous physical activity and much self reported stress were associated with an increased risk of ALS. The greatest effect on risk for ALS was posed by the combination of a type A behavior pattern and a less frequent intake of green-yellow vegetables, for either each gender or both. We observed a significant increased risk of ALS for women who had a less frequent intake of green vegetables without a type A behavior pattern.

Conclusion: Type A behavior pattern and a less frequent intake of green vegetables were independently associated with an increased risk of ALS for each gender, and marked gender-differences in the relationship of type A behavior pattern alone or a less frequent intake of green vegetables alone to the risk of ALS were observed. This suggests that gender-specific primary prevention of ALS based on gender-differences in imbalances between the excessive production of oxidants originating from patient-specific factors and the decrease in or lack of an antioxidant system in motor neurons may be more important.

P123 LIFETIME ESTROGEN EXPOSURE AND THE RISK OF AMYOTROPHIC LATERAL SCLEROSIS

DE JONG S[1], VELDINK JH[1], HUISMAN M[1], SUTEDJA N[1], VAN DER KOOI A[2], DE VISSER M[2], SCHELHAAS J[3], VAN DEN BERG LH[1]

[1]*Department of Neurology, Rudolf Magnus Institute of Neuroscience University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Department of Neurology, Academic Medical Center, Amsterdam, Netherlands,* [3]*Department of Neurology, Radboud University Medical Center, Nijmegen, Netherlands*

E-mail address for correspondence: sjong@umcutrecht.nl

Keywords: estrogens, risk, population-based

Background: The pathogenesis of amyotrophic lateral sclerosis is considered to be multifactorial. Several epidemiological studies showed a lower incidence of ALS in women than in men, most distinctively before menopausal age. These results suggest a possible protective effect of female reproductive hormones, especially endogenous estrogens.

Objectives: The aim of this study was to establish the relationship between endogenous estrogen exposure and ALS in a large and representative, prospectively collected patient group compared to population-based controls.

Methods: We performed a population-based case-control study in the Netherlands. Patients were diagnosed according to the established El Escorial criteria. Multivariate logistic regression analysis was performed in patients diagnosed with ALS after January 1st, 2006. Population-based controls were mainly recruited through the general practitioner of the patient. Patients and controls were sent a questionnaire. Data on reproductive history were collected. Only women with a natural menopause were included.

Results: Data from 131 incident patients and 430 controls were analysed. Multivariate analyses showed an increased risk of ALS in women with a higher age at menarche (OR 1.16 (95% CI 1.01–1.33)) and a shorter menarche-menopause interval (OR 0.95 (95% CI 0.91–0.99)). Also a lower body mass index before disease onset was associated with an increased risk of ALS (OR 0.92 (CI 95% 0.87–0.98)). A lower lifetime exposure to estrogens is associated with a worse prognosis (HR 0.93 (95% CI 0.88–0.98).

Discussion: This study provides evidence that a lower level of endogenous estrogen exposure and a lower body mass index are independently associated with an increased risk of ALS. Also, a lower level of endogenous estrogen exposure is associated with a worse prognosis.

Conclusion: This study provides evidence that a lower level of endogenous estrogen exposure and a lower body mass index are independently associated with an increased risk of ALS. Also, a lower level of endogenous estrogen exposure is associated with a worse prognosis.

P124 INFLUENCE OF PHYSICAL ACTIVITY IN THE EVOLUTION OF ALS

RODRIGUEZ DE RIVERA FJ, SANZ GALLEGO I, OREJA GC, DIEZ TE

ALS Unit, Hospital Universitario La Paz, Madrid, Spain

E-mail address for correspondence: rodriguezderivera@yahoo.es

Keywords: physical activity, evolution

Background: Previous studies tried to find a causal relationship between intense physical activity and the development of ALS without success. Nevertheless it has not been studied if these patients were evolving differently than the rest of the ALS patients.

Objectives: Our aim is to evaluate if there are differences in the evolution of patients with an intense physical activity during the first 2 years after the diagnosis.

Methods: A descriptive study of consecutive patients attended from the moment of diagnosis with quarterly reviews in the ALS Unit from 2006 to 2010 was done. The age of onset, the initial localization of the symptoms, the worsening of the functional state (scale ALSFRS-r), the respiratory function, the appearance of dysphagia and signs of depression and/or signs of Frontotemporal dementia were evaluated in relation to intense physical activity.

Results: Forty two patients (30 M and 12 W) were evaluated with an average age of onset of 57.97 years old. Eleven of these patients (10 M and 1 W with an average of age of onset of 47.45 years old) had precedents of an intense physical activity (7 sportsmen, 2 military men, 2 others) (Patients with Physical Activity (PPhA vs NoPhA)). The initial form was spinal in 81.81% and bulbar in 18.18% (NoPhPA S 61.29%; B 38.7%). The worsening of the functional state (PPhA 45.27 points ALSFRS-r at the beginning to 22.88 at 24 months (−22.39 pts) vs NoPhA 41.58 to 17.17 (-24.41pts)), the need of Non-Invasive Ventilation (44.44% vs 53.57%), the presence of dysphagia (33.33% vs 70.96%), signs of depression (36.36% vs 70.96%) and signs of frontotemporal dementia (27.27% vs 35.48%) were significantly less at 24 months of evolution in the PPhA patients. There were no statistically significant differences in the mortality.

Conclusions: Patients with intense physical activity are young males and their initial symptoms were predominantly spinal. PPhA patients have a slow clinical deterioration compared to other ALS patients during 24 months after diagnosis, though there are no significant differences in the mortality.

P125 SMOKING, ALCOHOL AND THE RISK OF AMYOTROPHIC LATERAL SCLEROSIS: A POPULATION-BASED STUDY

DE JONG S[1], VELDINK JH[1], HUISMAN M[1], SUTEDJA N[1], VAN DER KOOI A[2], DE VISSER M[2], SCHELHAAS J[3], VAN DEN BERG LH[1]

[1]*Department of Neurology, Rudolf Magnus Institute of Neuroscience University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Department of Neurology, Academic Medical Center, Amsterdam, Netherlands,* [3]*Department of Neurology, Radboud University Medical Center, Nijmegen, Netherlands*

E-mail address for correspondence: sjong@umcutrecht.nl

Keywords: smoking, alcohol, population-based

Background: The pathogenesis of amyotrophic lateral sclerosis (ALS) is considered to be multifactorial and smoking has previously been posed as a possible risk factor in ALS. The level of education and alcohol consumption are assumed to be associated with cigarette use, and are therefore potential confounders. Previous studies yielded conflicting results due to methodological limitations and large population-based studies could strengthen the evidence.

Objective: The aim of this study was to establish the relationship between smoking and ALS in a large and representative, prospectively collected patient group compared to population-based controls.

Methods: We performed a population-based case-control study in the Netherlands. Patients were diagnosed according to the established El Escorial criteria. Multivariate logistic regression analysis was performed in patients diagnosed with ALS after January 1st, 2006. Population-based controls were mainly recruited through the general practitioner of the patient. Patients and controls were sent a questionnaire. Data on lifetime history of smoking, level of education and alcohol consumption were collected.

Results: Data from 494 incident patients and 1599 controls were analysed. Multivariate analyses showed an increased risk of ALS in current smokers (OR 1.38 (95% CI 1.02–1.88)) and those with a lower level of education (OR 1.94 (95% CI 1.31–3.08)) and a reduced risk of ALS in those with current alcohol consumption (OR 0.52 (95% CI 0.40–0.75)). Also, current smoking had a significant effect on survival with a hazard ratio (HR) of 1.51 (95% CI 1.07–2.15), adjusted for vital capacity, gender, age and site of onset and had a notably larger effect on survival in women (HR 2.12 (95% CI 1.25–3.61)). There was no interaction between smoking and alcohol use.

Discussion: We conclude this study provides evidence that cigarette smoking and a lower level of education are independently associated with an increased risk of ALS and alcohol consumption independently with a reduced risk of ALS. Also, current smoking is associated with a worse prognosis, especially in women and independently from forced vital capacity. Furthermore, this study shows that a suspected risk factor for ALS should be studied in an incident population-based patient group, when an effect on survival is also present.

Conclusion: Cigarette smoking and a lower level of education are associated with an increased risk of ALS and alcohol consumption with a reduced risk of ALS.

P126 ANALYSIS OF CO-MORBID CONDITIONS IN STUDIES OF AMYOTROPHIC LATERAL SCLEROSIS

WALLACE K, LANKA V, SHERMAN A, CUDKOWICZ M

Massachusetts General Hospital, Charlestown, MA, United States

E-mail address for correspondence: avsherman@partners.org

Keywords: co-morbid conditions, prevalence, clinical trials

Background: Most studies on Amyotrophic Lateral Sclerosis (ALS) collect data regarding non-ALS medical conditions. Such data may elucidate the more common co-morbidities within an ALS subject population. Additionally, it may provide a 'timeline' that chronicles another condition's diagnosis relative to the subject's ALS diagnosis.

Objective: The purpose of this analysis is to better understand which co-morbidities exist within the ALS-diagnosed populations. Within this objective, we sought to: 1) determine which 'medical body systems' categories had the highest concentration of co-morbidities, 2) derive which disorders are more prevalent and still exist post-ALS diagnosis, and 3) perform a sub-analysis where temporality of co-morbidity diagnosis is known with respect to the ALS diagnosis.

Methods: Data were pooled from eleven studies conducted at Northeast ALS Consortium's sites. Subject data were included if there was a substantiated date of ALS diagnosis that could be linked to data in co-morbidities case report forms that provide the data elements of a 'Body System' category, description of a condition and whether the condition was present at screening. 'Year of Diagnosis' data element was required for the subject to be included in the sub-analysis. For the co-morbidity 'free-text' comparisons, standardized coding terminology from MedDRA and DSM-IV were used in coding algorithms. Microsoft Excel and SAS v9.1 were used for calculations.

Results: The overall analysis revealed that the most prevalent medical body system categories were 'Musculoskeletal', 'HEENT' and 'Autoimmune Disorders'. When examining only those co-morbid conditions that were present at screening, these included 'Mood Disorders', 'Hypertension' and 'Allergies'.
In the sub-analysis, 'Musculoskeletal' was also the most prevalent pre-ALS diagnosis medical body system category, followed by 'Genitourinary' and 'Cardiovascular'. The most prevalent pre-ALS diagnosis co-morbid conditions were 'Fractures', 'Major OBGyn with general anesthesia' and 'Cancer'. The most prevalent co-morbid conditions at screening were 'Mood Disorders', 'Sleep Disorders' and 'Generalized Body Pain'.

Conclusion: 1) A lot could be gained from analyses of co-morbid conditions, both prior to ALS diagnosis and post-diagnosis; 2) Recommendations could be developed for targeted interventions for subjects regarding co-morbidities; 3) Certain data collection improvements are desirable: Utilize standard data elements to collect co-morbidities across future ALS studies; Use medical data dictionaries (MedDRA, etc.) to code 'Medical Body Systems' categories; Introduce standard approach for analyzing 'free-text' format for co-morbid conditions.

P127 RE-WRITING HISTORY: LOCKHART CLARKE'S FORGOTTEN DESCRIPTIONS OF ALS FROM THE 1860S

TURNER M[1], SWASH M[2], EBERS G[1]

[1]*Oxford University, Oxford, United Kingdom,* [2]*University of London, London, United Kingdom*

E-mail address for correspondence: martin.turner@clneuro.ox.ac.uk

Keywords: history, neuropathology, pioneers

Background: It is accepted that others, including Charles Bell, François-Amilcar Aran and Jean Cruveilhier, had recognised a progressive lower motor neuron-only syndrome from at least the 1830s, before Jean-Martin Charcot's first use of the term ALS in 1874 (nine years after his first case report). Although the definitive term ALS, that acknowledged both upper and lower motor neuron involvement, is attributed to Charcot, William Gowers first grouped the three phenotypes of amyotrophic lateral sclerosis, progressive muscular atrophy and progressive bulbar palsy together as part of the same syndrome. The term MND as an over-arching label was not suggested until nearly a century later by W. Russell Brain.

The forgotten cases: Augustus Jacob Lockhart Clarke (1817–1880), a physician on the staff of the Hospital for the Paralysed and Epileptic in Regent's Park London, is best known for his descriptions of spinal cord anatomy. However two published and detailed case reports from the 1860s (pre-Charcot) have emerged in which he also carried out rigorous post-mortem neuropathological studies of what appear to be classical cases of ALS (1, 2). He clearly recognised the additional involvement of the corticospinal tracts that distinguished this from PMA, captured in beautiful drawings, and indeed his contribution was later acknowledged by Charcot.

As well as the painstaking pathological examination, exquisite clinical histories are documented in both cases, the first co-authored with Charles Bland Radcliffe, the other with John Hughlings Jackson. They resonate with contemporary debates concerning the evolution of disease in ALS. Both concern young-onset cases, the first with a military background and the onset attributed to sunstroke, the second apparently post-traumatic (and which later involved a successful pregnancy). The descriptions are graphic and specifically recognise features such as oculomotor, sphincter, sensory and cognitive sparing. Mercury and electrical stimulation were tried therapeutically, along with a diet of 'eggs, beef-tea, an extra allowance of bread, and half-a-pint of port wine...' to no avail.

Conclusions: The definition of the clinicopathological entity of ALS evolved over half a century, and Lockhart Clarke must take his rightful place among the other pioneers. His Lancet obituary stated: 'He was a man single of purpose, of noble independence and honesty, wholly free from ambition. . .he will be remembered, not as the popular physician, but on account of his patient and laborious researches so fruitful to medical science.'

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THEME 7 MULTIDISCIPLINARY CARE AND QUALITY OF LIFE

P128 RESTLESS LEGS SYNDROME IN AMYOTROPHIC LATERAL SCLEROSIS

PRALINE J[1,2], LIMOUSIN N[1], ARNULF I[3], BLASCO H[4], CORCIA P[1,2]

[1]*Centre SLA, Service de Neurologie et de Neurophysiologie Clinique, CHRU, Tours, France,* [2]*INSERM UMR 930, Université François-Rabelais, Tours, France,* [3]*Unité des Pathologies du Sommeil and UMR 975, Université Paris 6, Hôpital Pitié-Salpêtrière, Assistance Publique des Hôpitaux de Paris, Paris, France,* [4]*Laboratoire de biochimie et biologie moléculaire, CHRU, Tours, France*

E-mail address for correspondence: nadege.limousin@univ-tours.fr

Keywords: restless legs syndrome, sleep disturbance

Background: The restless legs syndrome (RLS) is a sensory-motor disorder severely affecting 2–3% of adults in western populations. It seems to be more prevalent than in the general population in specific neurodegenerative disorders including Parkinson's disease, Huntington's disease, spinocerebellar atrophy, and hereditary spastic paraparesis. No such prevalence data exists for RLS in ALS patients. If present, RLS could alter the quality of life in patients with ALS, and could be treated. Furthermore, ALS leads to complete immobility of the legs, a condition that can possibly exacerbate a pre-existing mild RLS. Consequently, it seems to be important to search for RLS in ALS patients as this symptom could be improved by treatment.

Objectives: To determine the prevalence and determinants of the association of RLS and ALS.

Methods: Consecutive, unselected ALS patients were recruited in our ALS centre. Information on sex, age of inclusion, age of onset, body mass index, duration of the disease, site of onset, ALS Functional Rating Scale Revised, use of percutaneous endoscopic gastrostomy tube, non invasive ventilation and tracheotomy was obtained. Each patient underwent a sleep interview. RLS was diagnosed using the IRLSSG criteria. Investigations looking for symptomatic RLS including iron and ferritin plasma levels were performed.

Results: A total of 69 subjects (69.6±9.7 years) were included. RLS was found in thirteen patients (18.8%). Most patients (92%) were assigned to the group of moderate or severe. Only 4 patients had previously been diagnosed as having RLS and were on medication. When excluding causes of symptomatic RLS, including iron deficiency (n = 1), sensory neuropathy (n = 1) and drug adverse effects (n = 3), 8 of 69 (11.6%) patients had a RLS without other causes. Patients with RLS had a worse score to the question "Has your ability to turn in bed and adjust the bed clothes changed?" than patients without it (P = 0.0004). No other significant difference about ALS features was found between these two groups.

Discussion: Our population survey was the first study about RLS prevalence in ALS patients and revealed a surprisingly high proportion of RLS. Progressive dysfunction of spinal axons is well known in ALS and could explain RLS. We discuss possible bias, especially the overlap between RLS and pain induced by immobilization.

Conclusion: RLS seems to be particularly frequent in ALS patients. An interview targeting RLS criteria should be performed in ALS patients with suggesting any sensory discomfort. The efficiency of dopaminergic agonists should be evaluated in ALS patients with coexisting RLS.

P129 ITALIAN VALIDATION OF AMYOTROPHIC LATERAL SCLEROSIS SPECIFIC QUALITY OF LIFE - REVISED (ALSSQOL-R): A COMPARISON BETWEEN U.S. AND ITALIAN SAMPLES

PAGNINI F[1,2], SIMMONS Z[4], FELGOISE S[5], LUNETTA C[3], CORBO M[3]

[1]*Department of Psychology, Catholic University of Milan, Milan, Italy,* [2]*Ph.D Program in Clinical Psychology, University o Bergamo, Bergamo, Italy,* [3]*NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Ospedale Niguarda Cà Granda, Milan, Italy,* [4]*Penn State University College of Medicine, Hershey, United States,* [5]*Philadelphia College of Osteopathic Medicine, Philadelphia, United States*

E-mail address for correspondence: francesco.pagnini@unibg.it

Keywords: quality of life, psychological assessment, cross-cultural comparison

Background: There is no consensus on how to measure quality of life (QOL) in amyotrophic lateral sclerosis (ALS). Most QOL instruments are heavily weighted toward strength and physical function, and will decline as ALS progresses. The ALS Specific QOL-Revised (ALSSQOL-R) questionnaire has been validated on 389 English-speaking ALS patients, but has not been used outside of the US. It has 6 subscales: 1) Negative Emotion; 2) Interaction with People and the Environment; 3) Intimacy; 4) Religiosity; 5) Physical Symptoms; 6) Bulbar Function.

Objective: 1) To determine whether QOL scores on the ALSSQOL-R differ in an Italian sample from norms originated on English-speaking US residents; 2) To determine if the 6 ALSSQOL-R factors demonstrate internal consistency and predict global QOL in the Italian population, as they do in English-speaking US residents.

Methods: One hundred and fifty ALS patients were recruited at NEuroMuscular Omnicentre in Milan and completed the Italian version of the ALSSQOL-R. Scores were compared to those from a US sample. Independent T-tests were used to

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compare subscale scores of US and Italian cohorts. Principal components analysis and Cronbach's alpha were used to measure factorial structure and internal consistency.

Results: The two samples were comparable with respect to age (Italian mean: 61.04 years; US mean: 60.5 years), gender (Italian: 40% female, US: 39% female), ALSFRS-R (Italian mean: 33.4; US: 33.11), employment status, and educational level. US scores were significantly higher in ALSSQOL-R total score ($P < 0.001$) and in the subscales of Interaction ($P < 0.001$), Intimacy ($P < 0.001$), Physical Symptoms ($P < 0.01$) and Bulbar Function ($P < 0.05$). There were no significant differences for Negative Emotion and Religiosity. The McGill QOL Single Item Score (MQOL-SIS) was significantly higher ($P < 0.05$) in the US sample. Principal components analysis with six factors explained 78.9% of the variance. All sub-scales demonstrated good internal consistency, with high Cronbach's Alpha: Negative Emotions (0.80), Interaction (0.81), Intimacy (0.70), Religiosity (0.84), Physical Symptoms (0.51) and Bulbar Function (0.84). ALSSQOL-R total score was positively correlated with MQOL-SIS ($P < 0.001$).

Discussion and conclusions: The US sample demonstrated higher overall QOL scores and higher subscores in 4 of 6 categories than the Italian sample, despite comparable demographics and functional measures. Factor analysis and measures of internal consistency indicate that the Italian version of the ALSSQOL-R has good psychometric properties, and appears to be a valid self-assessment screening tool in ALS care. The differences in normative values for some scales between the Italian ALS population and those in English-speaking US residents emphasises the need to independently test this instrument in ALS patients from different cultural backgrounds.

P130 CASE MANAGEMENT IN ALS: A NEXT STEP TOWARDS EXCELLENT CARE FOR PEOPLE WITH ALS?

CREEMERS H[1], VELDINK JH[2], GRUPSTRA H[1], NOLLET F[1], VAN DEN BERG LH[2], BEELEN A[1]

[1]*ALS Center The Netherlands/AMC, Amsterdam, Netherlands,* [2]*ALS Center The Netherlands/UMC Utrecht, Utrecht, Netherlands*

E-mail address for correspondence: h.w.creemers@amc.uva.nl

Keywords: case management, quality of life, needs assessment

Background: Studies have shown that the multidisciplinary ALS care is not always optimal, resulting in perceived problems by ALS patients, family and professional caregivers. The concept of case management has been suggested as an innovative strategy to optimize complex palliative care. We therefore wanted to investigate the potential optimizing effect of a case manager on quality of life of patients with ALS and the caregiver's burden by performing a cluster randomized trial. The case manager assesses the changing complex needs and preferences of the ALS-patients and their caregivers and arranges, in consultation and collaboration with the ALS care team, additional care and services.

Objectives: To report on the issues faced by ALS patients and their informal caregivers for which they consulted the case manager, and the related services and support provided by the case manager.

Methods: The randomized controlled trial included 132 ALS patients and their caregiver who received treatment from ALS care teams at 31 different institutions from all over the country. 67 patients received case management in addition to usual care (intervention group).

The case manager visited the participants at the start of the study and every three months at home. During these home visits participants were invited to share experiences, ask questions or raise issues that were problematic for them or they wished to be informed about.
The starting point was the questions of the participants. Between the home visits support from the case manager was provided via e-mail and telephone.

Results: Participants differed in their need for information, support, treatment, resources and care. Assessment and review of problems faced by ALS-patients and their caregivers regarding symptoms of ALS rarely resulted in actions of the case manager as treatment options provided by the ALS team were adequately considered and participants were on average satisfied with the approach of the ALS team.
Actions of the case manager were specifically in the area of emotional well-being by providing a listening ear for participants, discussing the impact of having ALS and the impact of symptoms on daily functioning. Other ALS-related issues were also raised frequently, eg the provision of assistive products and technology, advance directives, supporting children, respite care, power of attorney.
The number of services and support provided by case management is partly determined by the personal situation of the ALS patient, the stage of the disease, the progression of weakness, the support from the environment and the satisfaction with the offered care and treatment.

Conclusions: Comprehensive case management in ALS may optimize the complex care by providing emotional and practical support to people with ALS and their caregivers. Case management is highly intensive individualized care and starts with identifying cases in need of intensified management.

P131 PATIENT CARE COORDINATION AT CAROLINAS NEUROMUSCULAR/ALS – MDA CENTER: ALS CLINIC STAFF WEEKLY MEETING ACTION DOMAINS

BLACK KJ, WILLIAMS NM

Carolinas Medical Center - Department of Neurology, Charlotte, NC, United States

E-mail address for correspondence: kathryn.black@carolinashealthcare.org

Keywords: ALS team communication, quality improvement, audit

Background: What is important in care coordination within multidisciplinary teams has not been fully studied. Delivery of services requires organization of patient care together with sufficient and timely information. Patients require access and navigation through the healthcare system and there has to be a needs assessment with communication of needs to key personnel (1,2).

Objective: Identify ALS clinic staff weekly meeting action domains discussed during patient care coordination of a large ALS Clinic in the Department of Neurology situated in the third largest public healthcare system in the United States - Carolinas Healthcare System.

Methods: Quarterly audits of ALS Clinic Staff Weekly meetings were performed. Action domains were based on patient needs and problem solving.

Results: New patients were reviewed weekly and 10% of the patients referred for the diagnosis of ALS had other diagnoses.

Patient care reviews included 4-13 weekly with additional 0-2 hospitalized or nursing home patient discussions weekly together with 0-3 hospice referral patient discussions weekly. Home visits (0-13 weekly) were reviewed. Safety measures were reviewed weekly including in-clinic falls which occurred in 4 patients annually. All ALS deaths were reviewed (8-18 annually). ALS Care Fund dispersals were reviewed quarterly. Chemotherapy, gastrostomy tube, ventilator and wound (decubitus, tracheostomy, etc) status were discussed at initiation and then at change of status in each patient. Advanced directives together with altered mental status were discussed at each post-multidisciplinary clinic conference monthly. Research study and clinical trial patients (9-17 weekly) were discussed for 5 clinical studies and 6 clinical trials.

Conclusions: Action domains: newly diagnosed (8.5%), misdiagnosed (0.7%), follow-up non-hospitalized (42.4%), hospitalized (5.2%), hospice (5.5%), home visit (34.6%) and deceased (3.0%) patients, were systematically reviewed weekly by the ALS Clinic Staff to identify new problems and outline implementation measures. ALS Clinic Staff weekly meetings provide the venue for improving quality of care (3) and improving communication with innovative techniques (4).

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P132 PATIENT CARE COORDINATION AT CAROLINAS NEUROMUSCULAR/ALS – MDA CENTER: IN- HOME SERVICES – HOME CARE COORDINATION

NICHOLS MS[1], WILLIAMS NM[1], BLACK KJ[1], WRIGHT KA[1], LANGFORD V[1], DUFFY KS[1], SANJAK MS[1], WARD A[1], O'NEIL M[1], WALGREN K[1], OPLINGER H[1], BOCKENEK WL[1,3], BRAVVER E[1,2], BROOKS BR[1,2]

[1]*Carolinas Medical Center - Department of Neurology, Charlotte, North Carolina, United States,* [2]*University of North Carolina-Chapel Hill School of Medicine Department of Neurology, Chapel Hill, North Carolina, United States,* [3]*Carolinas Rehabilitation, Charlotte, North Carolina, United States*

E-mail address for correspondence: mindy.nichols@carolinas healthcare.org

Keywords: home care, in-home services, altered mental status

Background: In-Home Services provide an extension of ALS care to specific patients who require additional attention between ALS multidisciplinary clinic visits. Some visits when supported by patients' insurance are provided through private home health services. When other visits are needed, help may be provided by patient service organizations such as the ALS Association and Muscular Dystrophy Association and local sources such as the Joe Martin Foundation. Only a limited number of ALS Clinics have a dedicated division providing in-home services.

Objective: Identify baseline activities provided by the in-home services division of a large ALS Clinic in the Department of Neurology situated in the third largest public healthcare system in the United States - Carolinas Healthcare System.

Methods: Home visits were audited quarterly and new problems identified by home visits categorized. Summary data was analyzed with descriptive statistics.

Results: Total annual contacts for one RN were 296 (152 males; 144 females). Home visits (97.3%) were the major contact while nursing home visits (2.7%) were less frequent. New problems (61.1%) were identified in home visits. The most common new problems (16.8%) were speech-augmented communication placement (8.5%) and swallowing-attention to gastrostomy tube (8.3%). The second most common problems (15.1%) were physical therapy and occupational therapy problems including falls (3.0%), new durable medical equipment (9.7%) and decubitus ulcers (4.4%). Next were respiratory problems (13.1%) comprised of drooling-hypersiallorhea (4.4%), ventilator issues (4.4%) and pneumonia-bronchitis (4.3%). Administrative problems (8.4%) included patients who were new to or missed recent clinics (5.6%) and benefit or hospice issues (2.8%). Altered mental status (5.7%) required nurse visits to identify the next course of action.

Conclusions: Three of five in-home service encounters by a dedicated in-home services division of an ALS Clinic identified new problems providing information that led to hospitalization (17.7%) with (5.2%) and without mortality. This audit identified that in-home service encounters comprise approximately 40% of the total combined intake clinic, multidisciplinary clinic, ventilator clinic and home visit encounters. This audit provides benchmark criteria for expanding in-home services when appropriate resources are available.

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Supported by: Carolinas ALS Endowment, Pinstripes Foundation, Carolinas Healthcare Foundation, Muscular Dystrophy Association/ALS Division.

P133 THE 'PREFERRED PRIORITIES FOR CARE' DOCUMENT IN AMYOTROPHIC LATERAL SCLEROSIS: VIEWS OF BEREAVED RELATIVES AND CARERS

PRESTON H[1], COHEN-FINEBURG I[2], CALLAGHER P[1], MITCHELL JD[1]

[1]*Preston MND Care and Research Centre, Royal Preston Hospital, Preston, Lancashire, United Kingdom,* [2]*International Observatory on End of Life Care, University of Lancaster, Lancaster, United Kingdom*

E-mail address for correspondence: hazzer9@hotmail.com

Keywords: Preferred Priorities for Care, end-of-life, advanced care planning

Background: Increasing emphasis is being placed on the need for advanced care planning (ACP) at the end-of-life. The Preferred Priorities for Care document (PPC), formerly titled the Preferred Place of Care document is a patient-held record, promoted by the UK Department of Health's End of Life Strategy introduced in 2007. As an ACP tool, it aims to promote discussion and communication of wishes and preferences amongst patients, family and health care providers at the end-of-life. Because the majority of patients with Amyotrophic Lateral Sclerosis (ALS) lose verbal communication yet retain the capacity to make decisions, early discussion of wishes, a central aspect of ACP, is particularly important. The congruence between actual and preferred place of death is becoming increasingly recognised as an outcome in evaluating the quality of end-of-life care and PPC is anticipated to help patients achieve their preferred place of death. However there has been little research evaluating PPC's effectiveness, or exploring user views, particularly in non-malignant disease.

Objectives: This study aimed to look qualitatively at ALS patients' bereaved relatives' experiences of using the PPC document and their perceptions about its impact on end-of-life care.

Methods: The research was conducted in consultation with the International Observatory on End of Life Care at Lancaster University. Participants were bereaved relatives or primary carers of patients with ALS known to the Preston MND Care and Research Centre in the North West of England, as identified by its database. Semi-structured face-to-face interviews were conducted at the participant's home and thematic analysis was used to analyse the written interview data.

Results: Eleven participants were interviewed and were mostly over 65 years, male, British White and living with the patient. Four main themes specific to the PPC document were identified: I) completion, II) document availability to others, III) importance and influence on end-of-life experience and IV) limitations.

Discussion: Completing the PPC was generally viewed as a positive experience for both participant and patient, affording peace of mind and a sense of control. It appeared less important as a communication aid between patients and participants, as a result of discussions held prior to its completion, than in raising awareness of patient preferences amongst Health Care Professionals (HCP's). Lack of awareness of the PPC amongst HCP's was identified by participants as the most important area for improving effectiveness of the document.

Conclusions: Participants felt the PPC to be an important document although its influence on the end-of-life experience was less clear. Importantly a perceived lack of awareness of the PPC document amongst HCP's has been highlighted. The implications for practice include looking at levels of and ways to improve awareness, particularly in light of the increasing pressure to achieve patient preferences at the end of life.

P134 THE MAPPA PROJECT: A LONGITUDINAL STUDY ON PSYCHOLOGICAL WELL-BEING OF ALS PATIENTS AND THEIR CAREGIVERS

PAGNINI F[1,2], LUNETTA C[3], ROSSI G[3], BANFI P[3], GORNI K[3], CELLOTTO N[3], MARCONI A[3], FOSSATI F[3], CASTELNUOVO G[1], CORBO M[3], MOLINARI E[1]

[1]*Department of Psychology, Catholic University of Milan, Milan, Italy,* [2]*Ph.D Program in Clinical Psychology, University of Bergamo, Bergamo, Italy,* [3]*NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Ospedale Niguarda Cà Granda, Milan, Italy*

E-mail address for correspondence: francesco.pagnini@unibg.it

Keywords: quality of life, longitudinal study, caregivers

Background: Quality of Life (QOL) and psychological well-being in ALS are an important clinical issue, for both patients and caregivers. These psychological variables are widely considered in cross sectional studies, but few works have investigated their evolution in longitudinal research.

Objective: We aim to evaluate how QOL, depression and anxiety change, during a period of one year, in ALS patients and their caregivers.

Methods: We conducted a repeated-measures study. Forty ALS patients, together with their caregivers, were assessed every 4 months (baseline, T1, T2 and T3). All subjects completed Beck Depression Inventory (BDI), McGill QOL Single-Item Scale (MG-SIS), State-Trait Anxiety Inventory (STAI) and caregivers also filled out the Zarit Burden Inventory (ZBI).

Results: We found a tendency of worsening in all analyzed constructs. Patient's MG-SIS gradually decreased over time, while BDI and STAI scores increased, with a statistical difference between every step's value, compared to previous assessment (T-test for related samples, P from <0.05 to <0.001). Caregivers also indicated a worsening of all psychological variables with a decrease of MGSIS and an increase of BDI, STAI and ZBI. Changes between times were higher for caregivers that for patients in all considered constructs.

Discussion: Psychological well-being of ALS patients and their caregivers gets worst over time. Considering the progressive nature of the disease, it seems that the advancement of physical impairment has a negative effect on QOL and promotes anxiety and depression in patients and caregivers, with an increase of burden for carers. Caregivers, in particular, get psychologically worse with disease progression. It is therefore important to offer psychological (sometimes psychiatric) support to both patients and carers, in order to reduce or contain the illness' effects on their psychological well-being.

Conclusion: The longitudinal design of our study indicates a worsening of psychological well-being indexes during time. This is true for ALS patients and even more for their caregivers that presented a greater worsening of psychological disease and a lowering of their QOL, compared to patients.

P135 SUPPORT SERVICES FOR LAY CARERS: VIEWS OF CURRENT AND PAST FAMILY CAREGIVERS OF PEOPLE WITH MND

O'BRIEN M[1], WHITEHEAD B[1,2], MITCHELL D[1], JACK B[1]

[1]*Edge Hill University, Ormskirk, United Kingdom,* [2]*Lancashire Teaching Hospitals NHS Foundation Trust, Preston, United Kingdom*

E-mail address for correspondence: obrienm@edgehill.ac.uk

Keywords: family carers, support, qualitative research

Background: Family caregivers play an important role in caring for people with MND. Little is known about the experiences of such family caregivers and the levels of support available to them or accessed by them. By developing an understanding of the concepts related to caring under these circumstances it should better enable us to meet the support needs of carers.

Objectives: To explore, from a qualitative perspective, the views of current and past carers of people with MND regarding their need for and use of support services.

Methods: Narrative interviews were carried out with a purposively selected sample of current (n = 18) and past (n = 10) carers of people with MND. Thematic analysis was aided by NVIVO 7.

Results: Consensus amongst participants was apparent in the themes identified within the data. Being a carer was described as extremely draining, both physically and emotionally. Despite the intense pressure and strain on carers, and the belief that respite care would make a difference to them and the person with MND, participants reported that such support was often not readily available.
Carers who had successfully accessed respite care regarded it as beneficial. However in some cases, where respite care was available, it was not accessed as carers felt guilty about leaving their partner in the care of someone else. Carers reported being unable to talk to friends and family about the impact of the disease on them and some expressed a wish to receive counselling. Those who had been able to access counselling services had found them beneficial. However, counselling was often not available or difficult to organise and some carers did not know how to access this service.
Some carers had accessed support from carers' groups, although not all found this met their expectations. Those who did not have access to a local support group felt this would be beneficial.
Support failed to address educational needs regarding care and equipment.

Conclusions: Exploration of carers' experiences in this manner has made it possible to arrive at a greater understanding of the challenges faced when caring for a family member with MND. It is apparent that there needs to be a review of support services provided for such carers as current availability is not only haphazard but also difficult to access at times. It should be noted that where support services are available individual circumstances may still preclude their uptake. It is evident from this study that one size does not fit all and what might be appropriate to one carer may be unacceptable to another. It is recommended that a range of support services be made available, with ease of access, from which carers can select those most appropriate for their individual needs.

P136 SOCIAL CARE AND OUT OF POCKET DISEASE COST IN PATIENTS AND FAMILIES WITH AMYOTROPHIC LATERAL SCLEROSIS IN SPAIN

MORA JS[1], SALAS T[1], MARÍN S[1], MORÁNY[1], FAJARDO ML[2]

[1]*ALS Unit Hospital Carlos III, Madrid, Spain,* [2]*Epidemiology Unit Regional Health Authority, Huelva, Andalucia, Spain*

E-mail address for correspondence: jmora.hciii@salud.madrid.org

Keywords: social-care, financial cost, Spain

Background: Suffering an adult onset progressively disabling disease such as ALS may produce a significant decrease in family income caused by changes in the provider roles of patients and caregivers, as well as a significant increase in expenses for health and social care not appropriately provided by the public system. There is not yet data on this subject in ALS care in Spain, where there is a public health care system.

Objectives: To know the status of social care and economic burden on Spanish ALS patients and families in order to specify needs and to propose improvements.

Methods: We created a 156 item questionnaire that included demography, clinical status and administered health care. An additional 61 questions covered labour status, social aids and out of pocket economic cost of the disease. The questionnaire was hand delivered or mailed to up to 400 patients with ALS from the Spanish ALS Foundation nationwide. Response to date is 260. A univariant analysis with SPSS 10.0 for Windows was performed.

Results: The patients' median profile is male/female (ratio 1:1); 54 years old (range 29-79); married (81%); spouse as primary caregiver (70.8%); and from Madrid (41.5%). Specific situations related to social care and disease cost were identified. Among them, 6.7% of patients have a full-time job versus 51.7% prior to the disease. 45.9% of caregivers were reduced to a part-time job. Median delay in the official recognition of disability was 11.4 months; 5.2 additional months to receive financial aid. These delays negatively influence the recognized degree of disability and the aid obtained. 38.7% of patients depend on this aid, and 33.9% on spousal income. During the last 12 months the aid received by 67.7% of patients was less than 3,000 Euros; and over the next 12 months 69.6% of families expect to receive less than 6,000 Euros. Family cost of the disease varies from 10,000 to 60,000 Euros per year depending on clinical status, knowledge of needs and available aids, and family economic resources. Detailed data will be presented.

Conclusions: Social aid is delayed and insufficient. Family expenses and required needs are often higher than the aid received and the family total income. A more agile processing of the disability recognition and an increase in financial aid are necessary. The recent approval of a Dependency Law to finance caregivers should help to lessen the economic burden of the disease, although the current financial crisis delays the practical application of the law.

P137 ALS PATIENTS ON A UNIVERSITY PALLIATIVE CARE UNIT – DEMOGRAPHIC AND CLINICAL DATA

LORENZL S, HENSLER M

Department of Neurology and Palliative Care Unit, LMU, Munich, Germany

E-mail address for correspondence: stefan.lorenzl@med.uni-muenchen.de

Keywords: university palliative care unit, symptom control

Purpose: We have evaluated the demographic data of admissions of ALS patients to our palliative care unit at the University of Munich, Campus Grosshadern from January 2007 until December 2009.

Methods: Retrospective analysis of patients' charts.

Results: In 2007 there were 12 patients (4% of all patients) with ALS (m:w = 6:6). The mean duration of hospitalization was 11±6 days. Four patients had a second stay (12±4 days). Five patients died during their time on our palliative care unit. In 2008 there were 14 patients (5 %) with ALS (m:w =7:7). The mean duration of hospitalization was 9±7 days. One patient had a second stay (7 days). Seven patients died during their time on our palliative care unit. In 2009 there were 10 patients (3 %) with ALS (m:w = 5:5). The mean duration of hospitalization was 11±6 days. None of the patients had a second stay. Three patients died during their time on our palliative care unit.
The main reasons for admission have been pain, respiratory insufficiency/depression and swallowing problems as well as increasing weakness. There has been no request for hastened death during the observation period. Two patients have been admitted to a hospice, but the majority have been able to get home again.

Conclusions: Although the number of patients with ALS admitted to our palliative care unit is relatively low it has been almost stable over three years. However, the duration of the hospitalization tends to decrease with admission of patients who are severely ill and die at the unit. Admission to a palliative care unit has not been associated with the wish to hasten death during this period.

P138 DEVELOPMENT OF AN INTERACTIVE SOFTWARE FOR EVIDENCE BASED SYMPTOMATIC MANAGEMENT OF MOTOR NEURON DISEASE WITH SPECIFIC TRIGGER POINTS TO SPECIALIST PALLIATIVE CARE INTERVENTION

BEDE P[1,2], OLIVER D[3], O BRANNAGAIN D[4], HARDIMAN O[1,2]

[1]*Trinity College, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland,* [3]*University of Kent, Rochester, United Kingdom,* [4]*Dochas Centre, Drogheda, Ireland*

E-mail address for correspondence: bedepeter@hotmail.com

Keywords: palliative care, symptomatic management, software

Background: The optimal management of Motor Neuron Disease (MND) requires a palliative approach from diagnosis; maintaining quality of life for patients and carers with effective symptom control and respecting patient autonomy. While the management of specific symptoms is well described in the literature, the timing and triggers to specialist palliative care intervention are poorly defined. Frequently specialist palliative care referral only happens in the terminal phase of the disease. We propose a flexible model of episodic, consultation based palliative framework from the diagnostic stage of the disease.

Methods: Based on a literature review of current international guidelines on palliative care intervention in MND we have developed a computerised interactive guide to help physicians and general practitioners to consider evidence based interventions at various stages of the disease. The programming language Object Pascal has been used in the development of the software.

Results: A user-friendly software using a series of simple dropdown menus has been developed giving evidence based recommendations depending on the severity of the following complaints: Dyspnoea, Dysphagia, Dysarthria, Sialorrhea, Pseudobulbar affect, Fatigue, Cognitive and behavioural deficits, Musculoskeletal pain, Sleep disturbances, Muscle weakness, Functional decline, Psychological distress and End of life decisions. The proposed software suggests referral to specialist palliative services based on symptom severity. A number of rating scale calculators and assessment algorithms are also included.

Conclusion: The proposed program is intended as an interactive aid to consider management options and specialist palliative care referral at the various stages of Motor Neuron Disease.

P139 UNILATERAL PAROTID ELECTRON RADIOTHERAPY AS A PALLIATIVE TREATMENT FOR SIALORRHEA IN AMYOTROPHIC LATERAL SCLEROSIS

HODSKINS J, ST. CLAIR W, KASARSKIS E

University of Kentucky College of Medicine, Lexington, KY, United States

E-mail address for correspondence: jshods2@uky.edu

Keywords: sialorrhea, radiation, parotid

When ALS patients experience some degree of bulbar involvement, sialorrhea can become a considerable challenge. Drooling has a profound negative impact in ALS patients' quality of life with embarrassing social implications. Several therapeutic modalities, including anticholinergic drugs, botulinum toxin injection, and radiotherapy have emerged as treatments for drooling in ALS. This retrospective case-series study examined the effect of palliative radiotherapy in controlling problematic oral secretions in 5 ALS patients refractory to medical management. In this series, external beam radiation with 500 cGy electrons was targeted to the parotid gland unilaterally at a depth determined by 3D CT scanning. Two patients received additional doses to the contralateral parotid due to persistent secretions. All patients reported some degree of benefit, resulting in a reduction in intensity and amount of drooling. There were no major side effects of treatment. We conclude that low-dose, conservative electron radiotherapy directed to the parotid glands provides satisfactory relief from sialorrhea in ALS patients and should be considered as a therapeutic option for patients refractory to medical management.

P140 SPEECH THERAPY AND COMMUNICATION DEVICES: IMPACT ON QUALITY OF LIFE IN PATIENTS WITH ALS

SCHMALBACH S, SIENIAWSKI M, KOLLEWE K, DENGLER R, KRAMPFL K, PETRI S

Department of Neurology, Medical School Hannover, Hannover, Germany

E-mail address for correspondence: schmalbach.sonja@mh-hannover.de

Keywords: speech therapy, communication device, quality of life

Background: Bulbar weakness is the first and main presenting feature in approximately 25 to 30% of patients with motor neuron disease (MND). The degeneration of brainstem motor nuclei results in dysarthria and dysphagia. The loss of speech as a result of dysarthria is one of the most profound changes that a patient with MND will experience. It is very distressing not only due to the resulting inability to communicate but also due to the loss of expression of personal characteristics such as humour and emotions.

Objectives: The aim of the present study was to investigate the impact of speech therapy and communication devices on the quality of life in patients with Amyotrophic Lateral Sclerosis (ALS).

Methods: ALS patients (n=30) with dys- or anarthria, who underwent speech therapy and/or used communication devices participated in the survey. They filled in three standardized questionnaires (Beck depression inventory (BDI), SF-36 questionnaire, revised ALS functional rating scale (ALS-FRS-R)) and were further interviewed about their experience with and benefit of speech therapy and communication devices.

Results: Results confirmed that loss of speech is a very high burden for patients. The effect of speech therapy on quality of life was only rated as moderate by most patients. In contradiction the prescription of a communication device mostly generated a considerable retrieval of quality of life, although initial troubles with the handling were often reported. We could further verify that information for patients about adjuvant methods is often poor if they do not attend specialized MND centres.

Discussion and conclusions: The inevitable decline in speech intelligibility in patients suffering from ALS with bulbar involvement can only marginally be delayed by speech therapy which might explain the only moderate impact on quality of life. When patients suffer from advanced dysarthria/anarthria, the use of a communication device can restore the ability to communicate, which was in general rated as having a great effect on quality of life.
In conclusion, informing patients about adjuvant methods like speech therapy and communication devices is very important, which highlights the significance of treatment in specialized MND centres. For maximum benefit, patients must be professionally instructed in the use of the particular communication device.

P141 POWER WHEELCHAIR MOBILITY OVER TIME IN ALS/MND: PSYCHOSOCIAL IMPACT, FUNCTIONAL USAGE, FALLS, PAIN RELIEF, AND COST

WARD A[1], GORELIK R[2], BROOKS BR[1], MITSUMOTO H[2]

[1]*Carolinas Medical Center, Charlotte, NC, United States,* [2]*Columbia University Medical Center, New York, NY, United States*

E-mail address for correspondence: amber.ward@carolinashealthcare.org

Keywords: adaptive equipment, assistive technology, quality of life

Background: Preliminary retrospective studies have been completed, and this survey furthers the information gathered to include expanded surveys and a psychosocial scale for more information on this population.

Objectives: To determine how the wheelchairs/features are used over time, the psychosocial impact of using a power wheelchair, information about pain and falls and cost/value information.

Methods: A questionnaire and the Psychosocial Impact of Assistive Devices Scale (PIADS) were sent to a consecutive sample of ALS patients who are power wheelchair users and are currently seen at one of two multidisciplinary ALS clinics; 35 questionnaires were returned. The surveys were sent out at one month and 6 months to yield information over time.

Results: One hundred percent of respondents felt their quality of life and overall mobility had improved since getting the power wheelchair. Twenty-five percent reported falls even after getting their chair, and no patients reported falling out of their chairs. All patients reported improvements in amounts of pain and lower extremity edema since getting the chair. All respondents reported sleeping for 1-2 hours in their chairs. We were able to compare usage of power features at 1 month and 6 months, and all respondents demonstrated increased usage of their chairs at 6 months. On the PIADS, all respondents reported the power chair had increased their competence, happiness, independence, quality of life, sense of control and ability to participate. Respondents noted an increase as well with their ability to adapt to activities of daily living, skillfulness and capability. Respondents also noted that having the power wheelchair decreased their embarrassment, confusion and frustration. Overall reported positive outcomes from having the chair, included items like freedom, safety, ability to go outside and longer distances, feeling less tired overall, feeling less dependent and having more ability to do daily tasks. Overall reported negative outcomes included being unable to visit inaccessible places, and that the power wheelchair was too big. No respondents had to delay Hospice to get their chair. The average time from onset of symptoms to wheelchair delivery was 48.6 months, and the time from diagnosis to wheelchair delivery was 38.4 months. The average cost of the power wheelchairs was $26,949, the average amount of wait time from evaluation to chair was 2.6 months, and from recommendation of the chair to delivery was 6.2 months.

Conclusions: We obtained first hand knowledge from 35 ALS patients who are current power wheelchair users, on their functional usage, falls, pain and edema management, psychosocial adjustment, cost and satisfaction with their power wheelchairs over time.

P142 POSSESSION OF ASSISTIVE DEVICES AMONG PERSONS WITH AMYOTROPHIC LATERAL SCLEROSIS

OTERA A, TAKAHASHI K, OGINO M, FUKUDA M

Kitasato University East Hospital, Sagamihara Kanagawa, Japan

E-mail address for correspondence: imazeki@kitasato-u.ac.jp

Keywords: occupational therapy, assistive devices, activities of daily living

Background: Amyotrophic lateral sclerosis (ALS), the progressive neurologic degenerative disease, has been shown to affect the person's ability to continue performing activities of daily living (ADL). Occupational therapists play an important role in prescribing appropriate assistive devices at appropriate timing to maintain their independence in ADL. There are some studies on the use of wheelchairs in persons with ALS. However there are only few studies looking at other assistive devices. Without any guidelines, the processes of selecting the assistive devices tend to depend on the experience of therapists or the advice from the mentor.

Objective: The purpose of this study was to describe the type and the timing of prescription of assistive devices such as orthoses, aids for eating, drinking and communication in persons with ALS.

Methods: The participants in our study were 97 persons with ALS/MND who were receiving occupational therapy at our hospital from April 2003 to March 2009 (57 men and 40 women; age range, 30-82 y). ALS onset types were either of upper type (46%), lower type (27%), or bulbar type (24%).
We retrospectively looked up the clinical recording for the past six years, and researched the type of assistive devices and the timing of the prescription.
The data was analyzed according to the following four points: 1) Assistive devices were grouped into five categories according to the criteria determined by the association of technical aids in Japan. We then accounted the rate of possession for each category; 2) In terms of ALS onset type; 3) In terms of the period of onset; 4) In terms of the score on ALSFRS-R.

Results: The report showed that 65% of the participants in our study (n = 63) were prescribed at least one assistive device. The average number of devices in those 63 participants was 2.8 (median = 3.0). 1) Rate of possession for each category of assistive devices were: cervical collar (13%), wrist-hand orthoses (32%), upper limb orthoses (non-body worn) (15 %), aids for personal care (5%), aids for eating and drinking (27%), computer (12%), sound transmission system (5%), face to face communication aids (31%); 2) Assistive devices were prescribed mostly in persons with upper type ALS. Cervical collars were prescribed in bulbar type. There was no peculiarity in other types of ALS; 3) There was no relationship between the type of the assistive devices and the period of onset; 4) When persons had a high score on ALSFRS-R, they took possession of orthoses and aids for personal care (eating and drinking). When they had a low score on ALSFRS-R, they took possession of computers and communication aids.

Discussion and conclusions: This study demonstrated the possession of the assistive devices among persons with ALS quantitatively.

P143 HYDROTHERAPY PROGRAM FOR PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS WHO HAVE LOST THE ABILITY TO WALK

SILVA T[1,2], CONCEIÇÃO E[1], CHAVES CA[1], CUNHA M[1,2], DURAN M[1,2], OLIVEIRA A[1]

[1]*UNIFESP–EPM, São Paulo, Brazil,* [2]*ABRELA, São Paulo, Brazil*

E-mail address for correspondence: tatimsilva@gmail.com

Keywords: hydrotherapy, walk

Background: The ability to walk in patients with amyotrophic lateral sclerosis tends to undergo changes due to the beginning of muscle atrophy; also later, foot equinus, as well as changes in balance. Although treatments carried out in swimming pools are widely used due to the benefits such treatment provides, there are very few studies which focus on the effects produced by body immersion. The heated water of a swimming pool facilitates initial gait training and balance.

Objective: Analyzing the starting time for gait loss in patients with sporadic and bulbar ALS.

Method: Fifty four patients with a diagnosis of ALS (42 with sporadic ALS and 12 with bulbar ALS) were accompanied for 3 years by a physiotherapy team from the Neuromuscular Disease sector of UNIFESP-EPM. Group 1 (SALS): 28 men and 14 women, mean age 55.5 years (28-83 years); group 2 (BALS): 2 men and 10 women, mean age 64 years (53-75 years). None of the 54 patients exhibited changes in their ability to walk during their initial assessment using item ´H` of the ALS-FRS Scale (Amyotrophic Lateral Sclerosis - Functional Rating). All 54 ALS patients were performing hydrotherapy once a week at a clinic-school. We analyzed the time when the first adaptations for walking were prescribed (use of anti-footdrop ankle orthosis, canes, or walkers), and when these patients lost their ability to walk and initiated use of a wheelchair. Patients were evaluated every 3 months.

Results: Fifteen months after diagnosis we verified that rehabilitation for SALS patients can increase the time needed for starting to use gait adaptations, and also after 24 months to increase the mean time for losing gait and starting the first adaptations. For BALS patients, 3 (12) lost gait after 19 months and 9 (12) developed changes for walking and started using a device to improve gait 20 months after diagnosis.

Conclusion: The findings suggest that rehabilitation can increase walking time and the ability to walk for SALS and BALS patients.

P144 FALLS IN MND: A REHABILITATION EXPERIENCE

TAYLOR E[1], RAYKAR V[2], DEWHURST E-K[1]

[1]*St Joseph's Hospital, Auburn, NSW, Australia,* [2]*Concord Hospital, Sydney South West Area Health Service, Concord, NSW, Australia*

E-mail address for correspondence: emma-kate.dewhurst@swahs.health.nsw.gov.au

Keywords: falls, injury, multidisciplinary care

Background: Falls are very common in older people and often under reported. It is estimated 1 in 3 Australians over 65 fall at least once per year and the incidence increases with increasing age (1). Significant injuries from falls, including fractures is reported to be approximately 2-6% (2). A fall is defined as 'an unexpected event in which the participant comes to rest on the ground, floor or lower level' (3).
People with MND have multiple risk factors for falls.
The MND service at St Joseph's Hospital services the western suburbs of Sydney, Australia. Newly diagnosed patients are admitted for a 2-3 day stay for purposes of assessment and early education. Patients are then seen in the multidisciplinary clinic every 3-4 months and are reviewed in the interim by any staff members as required.

Objectives: We reviewed the incidence of falls in people with MND, the injuries sustained and the consequences of those injuries.

Methods: We completed a retrospective review of the incidence of falls in people who attended our MND multidisciplinary service from 2007-2009 to review the incidence of falls, the incidence of injurious falls and the impact of those falls on function.

Discussion: Approximately 50 patients were seen by the MND service between 2007-2009. Of those patients, approximately 90% of patients had experienced at least 1 fall, with at least 50% of patients experiencing multiple falls.
We looked at the rate of injurious falls. Our definition of an injurious fall was a fracture – we did not record injuries such as superficial cuts and bruises. Approximately 10% of patients seen over this time had an injurious fall. 5 people had fractures that involved either the humerus, pelvis, fibula, patella or big toe. One person had 2 injurious falls, both resulting in lower limb fractures.
The consequences of these falls have been very significant. Not only did the injurious falls all require medical input, but 3 people required inpatient hospitalisation following the fall. The injurious falls had a significant impact on ADL performance and independence. The fractures following falls impacted mobility, activities of daily living, pain and problems with self-care and feeding and bed mobility.
This can also lead to an increase in carer burden.
We have recently implemented regular screening, falls prevention education and ensured the prescription of assistive aids.

Conclusions: Falls are very common in people with MND. Injury from falls has a significant impact on ADL's and carer burden in people with MND. Appropriate screening, education and use of prescriptive aids can prevent a proportion of these falls. Prevention of falls is a significant issue that needs to be addressed in people with MND.

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P145 MANAGING MEDICAL CRISES: THE EXPERIENCES OF PEOPLE AFFECTED BY MOTOR NEURONE DISEASE AND THEIR CARERS

HILL J

University of Nottingham, Nottingham, United Kingdom

E-mail address for correspondence: jackiehill@dsl.pipex.com

Keywords: out of hours, emergency services, medical emergency

Background: MND is a non-curative neurodegenerative disorder that has a rapidly progressive course. Due to the direct and indirect symptoms of the disease, people living with MND can suffer from a range of acute medical problems/emergencies throughout the course of their illness requiring a rapid response and proactive planning by professionals. The majority of people are supported and cared for at home and during an acute incident need to access primary care services and/or crisis intervention services ie out-of-hours doctors/nurses or ambulance services. There is limited knowledge and literature on the experiences of patients and carers of out-of-hours and ambulance services.

Objective: To explore the experiences of people affected by MND using crisis intervention services during a medical emergency.

Method: The research aim was met through a qualitative approach using in-depth, semi-structured interviews. Seven people with MND were interviewed, five primary unpaid carers of someone with MND and two bereaved carers.

Results: Three central themes were identified from the analysis of the data for participants with MND - independence, control and the quality of the crisis intervention services. The additional theme, guilt, was identified for carers.
From the themes discussed, three important aspects arose from this study: 1) Provision of information: Participants required information on how to manage practical aspects of the condition and to know who to ring in an emergency. They needed information on the systems, procedures and response times of out-of-hours and ambulance services; 2) Proactive management: In the study all participants with MND had experienced a fall, the results therefore highlighted the need for the provision of a key worker to provide proactive management of cases, support and information on services and advice on symptom management for people with MND and their carers; 3) Communication: The study identified a need for a system that enables the details of people with MND who have frequent falls to be 'flagged up' on the ambulance service computer system. The ambulance service lacked information on the crisis they were attending.

Conclusion: The study findings have highlighted a number of key areas for future research; in particular they suggest that the need to provide clear information to users of the ambulance service is important so that they understand and have confidence in the service. The study prompts a recommendation that work is carried out on the design and evaluation of an information leaflet on aspects of the ambulance service relevant to this patient group. An information leaflet will help reduce the anxiety felt by carers of people with MND and improve the quality of the care provided by the ambulance service.

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THEME 8 RESPIRATORY AND NUTRITIONAL MANAGEMENT

P146 EVALUATION OF RESTING ENERGY EXPENDITURE IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: COMPARISON BETWEEN METABOLIC MULTISENSOR ARMBAND AND INDIRECT CALORIMETRY

GALLETTI R[1], CHIÒ A[2,] SABET D[1], FADDA M[1], MANCINO MV[1], RIVETTI M[1], CALVO A[2], FINOCCHIARO E[1], BOGGIO BD[1]

[1]*Department of Clinical Nutrition, San Giovanni Battista Hospital, Turin, Italy,* [2]*Department of Neuroscience, University of Turin and San Giovanni Battista Hospital, Turin, Italy*

E-mail address for correspondence: rgalletti@molinette.piemonte.it

Keywords: malnutrition, resting energy expenditure, indirect calorimetry

Background: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease associated with dysphagia and consequent malnutrition. When oral feeding fails, enteral nutrition should be considered as an alternative or supplemental route for oral nutrition. It is therefore extremely important to strictly monitor patients' nutritional status.

Objectives: The aim of our study was to validate the measurement of Resting Energy Expenditure (REE) with a new tool called the Metabolic Multisensor Armband (SWA), using Indirect Calorimetry (IC) as the gold standard. We also measured body composition with a Body Impedence Analyzer (BIA), because we wanted to test the hypothesis that patients with ALS, while presenting a reduction of Fat-Free Mass (FFM), show an increase of metabolism.

Methods: After overnight fasting, we made a measurement of REE for 30 minutes using both IC (Calorimeter Delta Trak Datex Ohmeda) and SWA (SenseWear Pro2 Armband); then we measured body composition by BIA (Impedenzometry Tetrapolar Akern 101) and we calculated Fat Free Mass (FFM) and Fat Mass (FM). The results are presented as means and standard deviation; comparison between methods was assessed using the Bland-Altman test. Values were compared with Student's t test for paired data and with linear regression. A P level <0.05 was considered significant. The total number of patients was 39, 17 male and 22 female; 12 had bulbar onset and 27 spinal onset.

Results: The patients' mean age was 62.3 (±7.6) years, BMI 24.2 (±3.9), protein intake 62.5 (±13.7) g; nutritional blood tests were: total protein 7.2 (±0.4) g/dL; albumin 4.5 (±0.3) g/dL; transferrin 244 (±24.6) mg/dL; total cholesterol 190 (±36.3) mg/dL. The mean REE measured with DT was 1256 (±253) and measured with SWA was 1286 (±228) ($P = 0.64$). The two methods were highly comparable according to linear regression (DT vs SWA r^2 0.957; $P < 0.001$). The comparison with Bland Altman test demonstrates that SWA underestimated REE by about -28.3 calories compared to DT. Body composition differed from normality (taking into consideration patients' gender and age), with an increase of FM (59% in males and 60% in females) and a decrease of FFM (35% in males and 75% in females).

Conclusions: REE measurement with SWA was revealed to be accurate in predicting energy needs in patients with ALS at a non-advanced stage of disease. In our series, REE did not differ from normal controls.

P147 SAFETY OF HOME PARENTERAL NUTRITION IN PATIENTS WITH ALS: A FRENCH NATIONAL SURVEY

ABDELNOUR-MALLET M[1], VERSCHUEREN A[2], GUY N[3], SORIANI M-H[4], CHALBI M[1], GORDON P[1], SALACHAS F[1], BRUNETEAU G[1], LE FORESTIER N[1], LENGLET T[1], DESNUELLE C[4], CLAVELOU P[3], POUGET J[2], MEININGER V[1], PRADAT P-F[1]

[1]*APHP, CHU Pitié-Salpêtrière, Paris, France,* [2]*CHU La Timone, Marseille, France,* [3]*CHU Montpied, Clermont-Ferrand, France,* [4]*CHU Hôpital Archet, Nice, France*

E-mail address for correspondence: pierre-francois.pradat@psl.aphp.fr

Keywords: home parenteral nutrition, safety, radiologic inserted gastrostomy

Background: Gastrostomy is the most common alternative feeding device used in patients with amyotrophic lateral sclerosis (ALS) when oral intakes become inadequate. Percutaneous endoscopic gastrostomy (PEG) placement is not always possible in cases of severe respiratory failure. We carried out a retrospective multicentre study to assess the safety of home parenteral nutrition (HPN) in patients with ALS.

Patients and methods: We reviewed the case records of patients from French ALS centres treated with HPN by central venous catheter (CVC) using an implantable port between January 2005 and October 2009.

Results: Seventy-three patients received HPN for a total of 11908 catheter days. Twenty-seven patients experienced a total of 37 CVC related complications resulting in an incidence rate of 3.11 CVC complications /1000 catheter days, including 1.93 septic complications and 1.09 mechanical complications per 1000 catheter days. Metabolic complications were frequent but without serious consequences or mortality. The use of the catheter for intravenous therapies in addition to HPN was identified as a risk factor associated with a high rate of septicaemia (relative risk (RR) = 2.54, confidence interval CI (1.56;4.14), $P = 0.04$)

Discussion and conclusion: HPN is an alternative procedure to PEG in advanced ALS patients. The incidence of complications appears to be comparable to data from the literature on HPN in other diseases. A prospective study comparing HPN and radiologically inserted gastrostomy (RIG) would allow comparison of the relative risk-benefit and survival of these procedures. The relation of timing of CVC and RIG placement and the occurrence of complications should also be investigated.

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P148 BELIEFS AROUND PERCUTANEOUS ENDOSCOPIC GASTROSTOMY TUBE PLACEMENT IN ALS PATIENTS

HOSKINS K, DAHODWALA N, ELMAN L, GOLASZEWSKI A, PALOVCAK M, MCCLUSKEY L

University of Pennsylvania, Philadelphia, PA, United States

E-mail address for correspondence: katelin.hoskins@uphs.upenn.edu

Keywords: percutaneous endoscopic gastrostomy tube, attitudes, decision-making

Background: The progressive muscle weakness characteristic of ALS may make swallowing and eating difficult or impossible. Though patients may qualify for placement of a percutaneous endoscopic gastrostomy tube (PEG) based on impaired swallowing, weight loss, and deteriorating vital capacity, some elect not to undergo or to delay the procedure.

Objectives: We explored the attitudes and concerns of ALS patients considering PEG placement to better understand the decision-making process.

Methods: With guidance from patient and caregiver groups, experts from various clinical disciplines collaborated to create a 32 question survey assessing factors related to the decision-making process, PEG placement procedure, stress related to eating, quality of life, caregiver involvement, body image, and role of a PEG in prolonging life. Patients with a predicted forced vital capacity of 50% or less, weight loss greater than or equal to 5–10% body mass, and/or difficulty swallowing both liquids and solids were educated about the risks/benefits of PEG placement. Thirty-five consenting patients completed our survey. We assessed responses with a bivariate statistical analysis comparing subjects who replied that they were either very likely or likely to choose a PEG (Group 1) to those who were very unlikely, unlikely, or not sure (Group 2).

Results: Seventy percent of respondents classified themselves as very likely or likely to choose a PEG and comprised Group 1. Group 1 expressed little concern that a PEG would impede their ability to eat/drink by mouth. They were neutral or had little concern regarding post-op pain, infection, and mortality. Group 1 demonstrated significantly more stress related to eating, drinking liquids, and taking medications than Group 2, believing that PEG placement would reduce the stress of eating ($P = 0.0002$) and drinking ($P = 0.0007$). Group 1 also believed that a PEG would significantly reduce choking, as well as reduce caregiver concerns. Sixty-eight percent of participants believed that a PEG would reduce risk of aspiration pneumonia. Over half believed that a PEG would increase survival. Though both groups' quality of life means were comparable, Group 2 expressed greater concern that a PEG would extend their suffering with ALS. Group 1 believed that a PEG would likely improve their quality of life ($P = 0.0001$).

Discussion and conclusions: Current difficulty with eating, drinking, and taking medications plus the beliefs that a PEG would reduce the stress of eating and drinking, reduce patient and caregiver concerns, reduce choking on solids and liquids, as well as increase quality of life were all significant factors in the likelihood of electing PEG placement. Patients demonstrated misconceptions about the risks of aspiration pneumonia and the role of PEG in survival. More information is needed on the role of caregivers in the decision-making process. These issues should be incorporated into discussions surrounding possible PEG placement.

P149 NUTRITION AND RESPIRATION: COMBINING GASTROSTOMY WITH DIAPHRAGM PACING FOR IMPROVED SURVIVAL

ONDERS R, KATIRJI B, SCHILZ R, PONSKY J, ELMO M, KAPLAN C

University Hospitals Case Medical Center, Cleveland, Ohio, United States

E-mail address for correspondence: raymond.onders@uhhospitals.org

Keywords: gastrostomy, diaphragm pacing, PEG

Background: Since 1980 when the percutaneous endoscopic gastrostomy (PEG) was first described its role in ALS/MND was soon identified to help overcome weight loss and dehydration from dysphagia. Current AAN practice parameters state that PEG should be considered to stabilize weight and prolong survival. One of the major risk factors of PEG placement is further compromising respiratory function, which leads to a 30 day mortality rate approaching 25%. Diaphragm Pacing (DP) has over 10 years of use in spinal cord injured patients with pure upper motor neuron (UMN) involvement and PEGs. This report outlines the experience of DP and PEG in the combined UMN and lower motor neuron (LMN) involvement of the diaphragm in ALS/MND.

Objective: Analyze safety, utility and long term outcome of patients having simultaneous DP and PEG placement or utilizing DP during subsequent PEG placement.

Methods: Subgroup analysis of all patients who had DP and PEG placement during prospective, nonrandomized, controlled, pilot, and pivotal trials under IRB and/or FDA approval at a single institution as previously described.

Results: From 2005 until 2010, 17 patients had simultaneous DP and PEG and an additional 9 patients had PEG placement after DP implantation. The average age was 54 with 20 males and 6 females. Two patients were excluded: one due to an abdominal co-morbidity and one lost to follow-up. The FVC at either DP and PEG or PEG with DP was 53% predicted. There was no 30 day mortality. There were no perioperative pulmonary infections. The 6 month survival from PEG placement with DP is 88% and the one year survival 73%. The average post PEG follow-up to survival in patients who have diaphragm pacing is 29 months.

Discussion: Sedation given during PEG placement can suppress the respiratory drive in ALS/MND patients leading to posterior lobe atelectasis and increased risk of respiratory infections. This was identified by reading the continuous diaphragm EMG activity of the implanted electrodes during a PEG showing significant periods of diaphragm apnea which was overcome with DP use. By utilizing DP we showed an increase in respiratory compliance by 18% which decreases the work of breathing and makes recovery from the procedure easier. ALS/MND patients have an increased respiratory instability leading to apneas with oxygen therapy or pain medication that may be needed from the PEG. DP maintains respiratory drive in these patients and therefore decreases those risks. These factors may explain the improved survival compared to Forbes who reported 25% 30 day mortality and a year survival rate of 23% for PEG alone.

Conclusion: PEG with DP can be safely implanted simultaneously with an improved survival compared to historical experience of PEG alone.

P150 POSITIVE CLINICAL RESULTS OF DIAPHRAGM PACING IN ALS/MND WITH CHRONIC HYPOVENTILATION AND UPPER MOTOR NEURON RESPIRATORY DEFICITS WITH INTACT LOWER MOTOR NEURON PHRENIC MOTOR FUNCTION

ONDERS R[1], KATIRJI B[1], SO Y[2], KATZ J[3], MILLER R[3], NEWMAN D[4], SIMPSON E[5], APPEL S[5], BOYLAN K[6], MARAGAKIS N[7], LECHTZIN N[7], ROTHSTEIN J[7]

[1]*University Hospitals Case Medical Center, Cleveland, Ohio, United States,* [2]*Stanford University Medical Center, Palo Alto, California, United States,* [3]*California Pacific Medical Center, San Francisco, California, United States,* [4]*Henry Ford Hospital, Detroit, Michigan, United States,* [5]*Methodist Neurological Institute, Houston, Texas, United States,* [6]*Mayo Clinic, Jacksonville, Florida, United States,* [7]*John Hopkins Medical Center, Baltimore, Maryland, United States*

E-mail address for correspondence: raymond.onders@uhhospitals.org

Keywords: respiratory failure, diaphragm pacing, phrenic nerve

Background: Alternative therapies to help with respiration are needed in ALS/MND with a majority of deaths occurring in a median of three years from symptom onset from respiratory issues. In the United States, the Food and Drug Administration (FDA) has a separate pathway, humanitarian use device (HUD) designation, for approval of medical devices that affect fewer than 4000 patients per year. Safety and 'probable benefit' are analysed to allow access for patients with an orphan disease. The surgical safety and use of diaphragm pacing has been reported in ALS/MND. This report analyses the HUD designation subgroup of the DP database for whom diaphragm pacing will have long term probable benefit.

Objective: To report on the safety and probable benefit of DP in a post-hoc sub-group analysis of ALS/MND patients implanted in a prospective, nonrandomized, controlled, multicenter, interventional trial with a lead-in design and 9 month post -implantation treatment period with follow-up until death.

Methods: Subjects were included for analysis that had intact phrenic nerves as demonstrated by phrenic nerve conduction studies or clear visualization of diaphragm movement under fluoroscopy, who also had evidence of chronic hypoventilation by FVC $<$ 50% or MIP $\leq$60 cm, H_2O or $PCO_2 \geq$ 45 mmHg.

Results: Eighty six subjects out of the total 145 subject enrolment from 3/2005 to 1/2009 met the inclusion criteria for analysis. Four patients were further excluded from analysis due to co-morbidities (2) and being lost to follow-up (2). The remaining patients have provided survival follow-up data with over 120 cumulative total years of use with no patients stopping use of the device due to adverse device effects. Subject demographics include average age 58, 67% male, median onset to implantation 33 months, 33% bulbar onset, 80% riluzole use, 76% use of NIV, ALSFRS-r at implant 28 and FVC at implant 59% predicted. In the patients who had declining FVC during lead in there was a positive improvement in the decline of 1.14% per month (95% CI 0.29, 1.97, $P < 0.01$). There was an observed 30% improvement in the rate of decline of ALSFRS-r post implantation though not statistically significant except when analyzing the respiratory sub-score alone in which there was an improvement. Twelve patients had pre and post implant sleep studies showing a significant improvement in sleep quality. Forty patients have died and 11 more have had a tracheostomy with full time ventilation. None were related to device use. The one year tracheostomy-free survival rate from implant is 70% with a median of 20 months. The median tracheostomy-free survival from symptom onset is 58 months.

Conclusion: The DPS system can be safely implanted and utilized in ALS patients improving survival. The degree of intact nerve function to the diaphragm is the key factor in providing a successful symptomatic therapy.

P151 NON-INVASIVE VENTILATION (NIV) AND DIAPHRAGM PACING: DP AUGMENTS AND IMPROVES THE EFFECTIVENESS OF NIV IN ALS/MND LEADING TO AN IMPROVED SURVIVAL

LECHTZIN N[1], MARAGAKIS N[1], ROTHSTEIN J[1], ONDERS R[2], KATIRJI B[2], SO Y[3], KATZ J[4], MILLER R[4], NEWMAN D[5], SIMPSON E[6], APPEL S[6], BOYLAN K[7]

[1]*John Hopkins Medical Center, Baltimore, Maryland, United States,* [2]*University Hospitals Case Medical Center, Cleveland, Ohio, United States,* [3]*Stanford University Medical Center, Palo Alto, California, United States,* [4]*California Pacific Medical Center, San Francisco, California, United States,* [5]*Henry Ford Hospital, Detroit, Michigan, United States,* [6]*Methodist Neurological Institute, Houston, Texas, United States,* [7]*Mayo Clinic, Jacksonville, Florida, United States*

E-mail address for correspondence: raymond.onders@uhhospitals.org

Keywords: non-invasive ventilation, diaphragm pacing, NIPPV

Background: Non-invasive ventilation (NIV) can improve survival in patients with ALS/MND. Positive pressure ventilation however may suppress diaphragm function leading to atrophy and conversion to less functional fast twitch Type IIb from slow twitch Type I diaphragm muscle fibers (1). This report analyzes the prospective diaphragm pacing pivotal trial results with a retrospective cohort study of NIV use in ALS (2).

Objective: Compare NIV use alone to NIV and DP conditioning in ALS/MND.

Methods: The entire Lechtzin *et al's* data set that was used for a publication was made available so that a more direct comparison to the prospective, controlled, multicenter, interventional DP trial with lead-in and post -implantation treatment periods could be made. Analysis of inclusion criteria was then limited to FVC $<$ 65% predicted and $>$45% predicted to correlate with the DP study inclusion criteria. The DP patients analyzed also all met the criteria for chronic hypoventilation of FVC $<$ 50% or MIP $\leq$60 cm, H_2O or $PCO_2 \geq$ 45 mmHg. All patients in both groups needed to be using NIV.

Results: Forty four patients in the DP group met the analysis inclusion criteria of the 145 subjects enrolled with 106 implantations from 3/2005 to 3/2009. Forty three patients from the Lechtzin database met the criteria from a 298 patient database from 1998-2005. The DP/NIV group was younger than the NIV alone group (59 ± 9 vs. 64 ± 9, $P < 0.05$) and had a lower ALSFRSr score(28 ± 8 vs. 32 ± 7, $P < 0.05$) while the NIV alone group had a higher riluzole use (93% vs. 70%, $P < 0.01$) with more males (75% vs. 53%, $P < 0.05$). There was no statistical difference between DP/NIV and NIV alone group in the remainder of the comparison: bulbar onset (31% vs. 20%); G-tube use (61% vs. 53%); onset to diagnosis (17 ± 19 vs. 15 ± 11 months); diagnosis to first respiratory therapy (15 ± 13 vs. 11 ± 7 months); or FVC - % predicted (54 ± 6 vs. 55 ± 6). In the DP/NIV group patients initially started using DP for five 30 minute sessions a day and then increased based on patient presumed benefits. During diaphragm EMG analysis of implanted electrodes while the patient was on NIV, if NIV suppressed diaphragm activity the patients used DP whenever utilizing NIV. In the DP/NIV group the median survival from therapy was 20.7 months$\pm$1.8 while in the NIV alone group it was

11.9±2.2 (P < 0.001). The median survival from diagnosis was 37.5±3.5 in DP/NIV group and only 21.4±5.8 in NIV alone group (P < 0.001).

Conclusion: Although limited by the retrospective analysis, the results suggest a significant augmentive effect of DP pacing to NIV leading to improved survival with no safety concerns with simultaneous usage. The most likely reason for this is prevention of diaphragm activity suppression with DP use.

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P152 THE PREDICTIVE VALUE OF THE PHRENIC NERVE MOTOR RESPONSE FOR NON-INVASIVE VENTILATION

PINTO S[1], SCOTTO M[2], PINTO A[1,3], SWASH M[1,4], DE CARVALHO M[1,5]

[1]*Neuromuscular Unit, Instituto de Medicina Molecular, Lisbon, Portugal,* [2]*Department of Mathematics, University of Aveiro, Aveiro, Portugal,* [3]*Department of Physical and Rehabilitation Medicine, Hospital de Santa Maria, Lisbon, Portugal,* [4]*Department of Neurology, Royal London Hospital, Queen Mary School of Medicine, University of London, London, United Kingdom,* [5]*Department of Neurosciences, Lisbon, Portugal*

E-mail address for correspondence: mamedemg@mail.telepac.pt

Keywords: non-invasive ventilation, phrenic nerve motor response, hazard ratio predictive value

Introduction: Respiratory insufficiency is the main cause of death in Amyotrophic Lateral Sclerosis (ALS), but it is usually a late event. Non-invasive ventilation (NIV) improves survival and quality of life in ALS. We have evaluated the predictive value of several clinical features and respiratory tests indicating the need for NIV in our ALS population.

Methods: We evaluated 460 consecutive ALS patients followed in our Neuromuscular Unit over the last 7 years. The inclusion criteria were: follow-up in our unit; clinical progression; age at disease onset between 20 and 80 years; disease duration ≤ 18 months at diagnosis; phrenic nerve motor response (mean value of bilateral peak-to-peak amplitude - Mean PhrenAmpl) and predicted forced vital capacity (FVC). FVC and Mean PhrenAmpl were tested ≤ 2 months after diagnosis. Patients with other medical conditions, in particular respiratory disorders, were excluded. Criteria to adapt patients to NIV were respiratory symptoms associated with low FVC or abnormal nocturnal oxymetry. We tested the hazard ratio for predictive value for NIV adaptation of the following features and measurements: presentation (bulbar vs spinal form); age at onset; disease duration at diagnosis; ALS-FRS; FVC; Mean PhrenAmpl. We applied Kaplan-Meier survival curves and Cox proportional-hazards regression analyses.

Results: We included 138 ALS patients, 59 women, 57 with bulbar-onset, age at disease onset 61.2±10.9 yrs (mean±SD) and disease duration of 10.94±4.5 months. ALS-FRS was 31.45±4.5 (from 14 to 39), FVC 84.5±23.6% and Mean PhrenAmpl 0.49±0.25 mV (from 0 to 1.28) at study entry. All patients were followed before and after NIV. The following covariates were predictive of NIV: late onset ($P = 0.027$), low ALS-FRS ($P = 0.027$), low FVC ($P = 0.006$), and low Mean PhrenAmpl ($P < 0.001$). For each unit increment in ALS-FRS, FVC and Mean PhrenAmpl there was a relative risk decrease of 3.6% (CI:0.4-6.7%), 1.2% (CI:0.3-2%) and 86.3% (CI:65.5-94.6%), respectively. For each year increment in age at onset there was a relative risk increase of 1.02% (1.0-1.04%). Stratifying for the region of onset showed that Mean PhrenAmpl was significant for both groups ($P < 0.001$ and $P = 0.071$ for spinal and bulbar-onset patients, respectively), low ALS-FRS for spinal-onset patients ($P = 0.022$) and late age at onset ($P = 0.021$) for bulbar-onset subjects.

Discussion: Mean PhrenAmpl is a non-volitional respiratory test which is strongly predictive of the need for NIV in ALS patients, independently of the region of onset. These results indicate that motor unit loss in the diaphragm is a main factor determining respiratory symptoms in ALS. This test should be more extensively applied in this disease.

P153 A RANDOMIZED CROSSOVER TRIAL OF EARLY NONINVASIVE VENTILATION FOR ALS

LECHTZIN N, CLAWSON L, KIMBALL R, WIENER C

Johns Hopkins University School of Medicine, Baltimore, MD, United States

E-mail address for correspondence: nlechtz@jhmi.edu

Keywords: ventilation, clinical trial

Background: Respiratory complications are a frequent cause of death in ALS. Noninvasive ventilation (NIV) is the standard therapy for individuals with ALS and severe respiratory muscle impairment and has been shown to improve survival and quality of life. Several studies suggest that early use of NIV may slow the decline in lung function and improve survival.

Objectives: To determine if use of NIV in ALS can slow decline in forced vital capacity (FVC).

Methods: This was a randomized cross-over trial in which patients with a FVC > 60% of predicted were assigned to a three month period on NIV and a three month period off NIV. The goal was to enroll 66 patients.

Results: Sixteen patients entered this study. The mean age was 56.8±11 years, 82% were male, 88% were Caucasian, and 29% had bulbar onset disease. The ALSFRS score at baseline was 41.1±5. The mean FVC at study entry was 3.57±0.96 L (91.1% predicted. The mean FEV1 was 2.85±0.77 L (85.7% predicted). The mean maximal inspiratory pressure was -70 cm H_2O and the mean arterial PCO_2 was 40.1±4 mmHg. While there were no significant effects of NIV on gas exchange or quality of life, there was a trend toward less change in FVC on NIV. During the period on NIV the FVC increased by 0.1 liters but decreased by 0.2 liters off of NIV (P = 0.07). The $PaCO_2$ increased by 1.4 mmHg on NIV and it decreased by 0.44 mmHg off NIV (P = 0.44). Quality of life score were similar during the period on and off of NIV. On NIV the physical component of the SF-12 decreased by 3.5 points and off NIV the score increased by 3.8 points (0.17). The mental component of the SF12 increased by 2.2 points while on NIV and it decreased by 3.4 points during the period off NIV (P = 0.37).

Discussion: This study was severely limited by poor enrollment. Additionally the duration on and off of NIV was only three months. The subjects studied had good normal lung function and gas exchange at the start of the study and did not decline appreciably during the 6 months of the study. While there was a suggestion that FVC improved during the period on NIV, this was not statistically significant and therefore this study is negative with respect to its primary outcome.

Conclusions: This study showed that people with ALS and normal lung function can use NIV but given the small sample size we are unable to draw any firm conclusion about potential benefits of NIV in this population. A larger study should be conducted to answer this important clinical question.

P154 NURSE ROLE IN USE OF NIPPV COMPLIANCE AND EFFICACY DATA

ARMSTRONG J, CASEY P, ALLEN J, HELLER S, AJROUD-DRISS S, SIDDIQUE T, SUFIT R, WOLFE L

Northwestern University, Chicago, IL, United States

E-mail address for correspondence: jarmstrong@nmff.org

Keywords: non-invasive ventilation, nursing role for non-invasive ventilation, efficacy data for non-invasive ventilation

Background: The use of non-invasive positive air pressure ventilation (NIPPV) has benefits for people with ALS (1). Both quality of life, as well as quantity of life can be improved. However, there is not an ALS standard of care for management of NIPPV therapy, nor do we have a definition for the role of nursing regarding triage and therapy. NIPPV devices contain internal pressure monitors (pneumotachographs) that measure parameters of both compliance (numbers of hours used) and efficacy (minute ventilation, leak, respiratory rate, tidal volume, and apnea/hypopnea index). This data is available on current commercial home NIPPV devices. The role of the nurse coordinator, neurologist and/or pulmonologist monitoring this data has not been described in the literature.

Objectives: Review data collected from home NIPPV devices. Identify the tasks associated with review of this data as part of the function of the role of the nurse coordinator.

Methods: Compliance and efficacy data was obtained from a retrospective review of people with ALS using NIPPV. The routine for collecting the data is a minimum of three months, averages monthly. Changes to the device settings were made accordingly to physician interpretation and more frequently with reported symptoms. This information included how often data was obtained and how frequently that data influenced changes in therapy. Therapy was managed by the nurse coordinator under the direction of the Pulmonologist in the ALS Specialty Center.

Results: Review of the therapy decisions regarding NIPPV when made considering symptoms in question and recent triage history between patient and nurse. Case presentations will explain how simple problems can be managed (ie mask fitting causing high leak reading) to complex problems (ie trending high minute ventilation with increasing c/o shortness of breath led to investigating for pulmonary embolism).

Discussions and conclusions: Compliance and efficacy data interpretation can assist healthcare professionals in maximizing NIPPV therapy for people with ALS. Managing the shallow breathing index has proven helpful. Monitoring trends in the data can be useful and supports collection of serial data. Patients may benefit from monitoring data at a minimum of every three months, or as frequently as symptoms are reported.

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P155 PATTERNS OF ADHERENCE TO NON INVASIVE VENTILATION IN MOTOR NEURON DISEASE PATIENTS: A PROSPECTIVE STUDY

ANDO H[1], CHAKRABARTI B[2], ANGUS R[1], THORNTON E[3], YOUNG C[1,3]

[1]*Walton Centre for Neurology and Neurosurgery, Liverpool, United Kingdom,* [2]*University Hospital Aintree, Liverpool, United Kingdom,* [3]*University of Liverpool, Liverpool, United Kingdom*

E-mail address for correspondence: youngca@btinternet.com

Keywords: NIV, adherence, noncompliance

Background: Non-invasive ventilation (NIV) is increasingly being offered to Motor Neuron Disease (MND) patients. Studies report benefit in terms of survival with intervention and adherence to ventilation.

Objectives: In a longitudinal study patients were assessed both physiologically and psychologically prior to initiation of NIV and 3 monthly until death. Part of the aim was to map usage of NIV.

Methods: Thirty-five (24 male) MND patients were enrolled; 4 died before NIV was established and 3 remain under monitoring. Of 28 patients offered NIV, 11 declined and 17 were established on treatment. Ten patients (9 male, mean age 60 years) have 6 or more months follow up on ventilation. Six had bulbar symptoms at NIV commencement. All had evidence of nocturnal hypoventilation as assessed by continuous pulse oximetry - criterion used: greater than 30 minutes $< 90\%$ oxygen saturation. Adherence to NIV was assessed by analysis of the 'memory card' storage device in the ventilator at 4-6 months post initiation and at 10-12 months. Patients were recommended to use the treatment initially at night during sleep and for a minimum of 4 hours.

Results: At 4-6 months post initiation, the overall cohort used NIV for >4 hours nightly for a mean 65% (SD 32%) of nights with 3/10 patients using NIV >4 hours nightly for $>90\%$ nights. Bulbar patients were deemed less compliant than non-bulbar patients (54% nights v 81% nights>4 hour use). By 10-12 months, the remaining cohort ($n = 7$) used NIV for >4 hours nightly for a mean 78% (SD 36%) of nights with 4/7 patients using NIV >4 hours nightly for $>90\%$ nights; not significantly different compared with use at 4-6 months; $P = 0.16$. Bulbar patients were again deemed less compliant than non-bulbar patients (68% nights v 92% nights >4 hour use). Only 2 patients used the treatment every night.

Discussion: Use of NIV increases with disease progression, however our data shows that adherence to NIV is significantly less than recommended, despite patients being aware we were assessing use of NIV. Electronic recording allows assessment of usage and for noncompliance to be identified, allowing the issue to be discussed with patients. There is a need to understand and address the issues determining noncompliance with NIV if adherence is to be addressed and patients are to realise the full benefit of this intervention.

Conclusion: Adherence to NIV is poor in these MND patients and at a level that is of clinical significance.

P156 THE LIVED EXPERIENCE OF PATIENTS WITH MOTOR NEURON DISEASE WHO DECLINED OR DID NOT TOLERATE NON-INVASIVE VENTILATION

ANDO H[1], WILLIAMS C[4], ANGUS R[2], THORNTON E[3], CHAKRABARTI B[2], PIGGIN L[3], YOUNG C[1,3]

[1]*Walton Centre for Neurology and Neurosurgery, Liverpool, United Kingdom,* [2]*University Hospital Aintree, Liverpool, United Kingdom,* [3]*University of Liverpool, Liverpool, United Kingdom,* [4]*Hope University, Liverpool, United Kingdom*

E-mail address for correspondence: youngca@btinternet.com

Keywords: NIV, treatment acceptability

Background: Non-invasive ventilation (NIV) is known to be an effective intervention in motor neuron disease (MND) and is increasingly being offered to patients. The main benefits of NIV in MND patients are prolonged survival and resolution of symptoms of nocturnal respiratory insufficiency. However, it has been acknowledged that some patients do not tolerate a trial of NIV, while other patients decline a trial of NIV when offered.

Objectives: To follow a cohort of MND patients prospectively in order to determine what psychological issues are important in patients declining or failing to tolerate NIV, particularly those issues which contribute to treatment failure.

Methods: Thirty five (24 male) MND patients were enrolled, 4 died before NIV was established and 3 remain under monitoring. Of 28 patients offered NIV, 17 were established on NIV whereas 11 (8 male, average age 74.1yrs) have declined or failed to tolerate NIV: 7 intolerants, 3 early refusers without trial, and 1 late stage decliner. Of these 8 had limb onset and 3 bulbar; however 9 had bulbar symptoms at the time of trialling NIV. Semi-structured interviews were carried out with all the patients. The interviews were analysed using interpretative phenomenological analysis (IPA). One patient with only one interview was excluded from the analysis as the interview provided insufficient information to contribute. Triangulation by a respiratory physician and 2 psychologists was carried out on the interviews from 10 patients to examine emergent themes.

Results: Several key themes emerged: 1) personal perceptions of NIV consequence, 2) maintenance of self identity, 3) negotiation of the disease symptoms and 4) external influences. The first theme addresses perceived negative outcomes of NIV, consequently generating reluctance towards NIV, yet the issue appeared to be closely related to the second theme: self identity. Self identity was observed to have become vulnerable with MND as a result of physical deterioration and the change in patients' perception of self was further challenged by NIV. The third theme focuses on a discrepancy between the physician's recommendations and the patient's perceived need. The last theme addresses negative impact of experiences with healthcare services in the patient's decision-making. Similar themes were identified in those who declined and those who were intolerant of NIV after a trial of treatment.

Discussion: Thirty nine per cent of patients who were offered NIV either declined or did not tolerate the treatment. Subjective perception of MND and its impact on identity, and negative experience of healthcare services, are factors for decision-making regarding NIV use.

Conclusions: A psychological dynamic relating to NIV use was explored constructively using IPA. Understanding the implications of NIV for individuals is important if treatment is to be offered optimally and sensitively.

P157 ORAL SECRETION SCALE SCORE IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS IS ASSOCIATED WITH TOLERANCE OF NONINVASIVE POSITIVE PRESSURE VENTILATION, NEED FOR HOSPICE OR TRANSITION TO TRACHEAL POSITIVE PRESSURE VENTILATION AND SURVIVAL

CAZZOLLI P[1], BROOKS BR[2], LEWARSKI J[3], MCKIM D[4], CHATBURN R[5]

[1]*ALS Care Project, Canton, OH, United States,* [2]*Carolinas Medical Center Department of Neurology, Charlotte, NC, United States,* [3]*Invacare Corporation, Elyria, OH, United States,* [4]*Ottawa Rehabilitation Centre, Ottawa, ON, Canada,* [5]*Cleveland Clinic Foundation, Cleveland, OH, United States*

E-mail address for correspondence: PCazzolliRN@aol.com

Keywords: noninvasive positive pressure ventilation, oral secretions, hospice care

Background: Noninvasive Positive Pressure Ventilation (NPPV) may significantly prolong survival in ALS patients but severe bulbar impairment limits its use. A clinimetric scale (4 = normal automatic saliva swallow; no drooling; 3 = automatic saliva swallow decreased; infrequent drooling; 2 = conscious saliva swallow required, drooling upright-leaning forward, lip-blotting 4-6 /hr; 1 = difficult conscious secretion swallowing, frequent drooling any position, lip-blotting 12-30 /hr, intermittent suctioning; 0 = constant drooling requiring constant lip blotting, regular suctioning) quantifying ability to clear oral secretions from the airway was developed based on a set of ALS patients followed in the home setting.

Objective: To develop and validate an Oral Secretion Scale (OSS) to predict tolerance of NPPV, the need for hospice (end-of-life) care, when to transition to Tracheal Positive Pressure Ventilation (TPPV), if desired, and survival of NPPV users with ALS.

Design and methods: In this observational study, 157 consecutive ALS patients were followed prospectively at the start of NPPV and for up to 40 months during subsequent home visits. Median survival was compared using Kruskal-Wallis One Way ANOVA; P values < 0.05 were considered significant.

Results: At the start of NPPV, 86/157 (55%) patients had no impairment (OSS score = 4). In 22/157 (14%) NPPV was attempted during acute respiratory failure but failed in 80% of those patients with OSS scores of 0 or 1. Median survival of 135/157 patients who continued on NPPV was significantly ($P = 0.002$) longer (27 months) in those with higher OSS score (= 4) and shorter (8 months) in those with lower OSS score (= 1). In 49/135 patients who stopped NPPV, median OSS score (=2) at the start of NPPV was significantly ($P < 0.05$) higher than the median OSS score (=1) when NPPV was no longer tolerated or when NPPV was stopped due to death, or transition to TPPV. Patients with an OSS score (=4) remain on NPPV more than 3 times longer than patients with OSS score (=1).

Conclusions: OSS score was associated with tolerance of NPPV, when to initiate TPPV and survival. OSS score of 1 reliably signaled the intolerance of NPPV and/or the need for transition to tracheostomy ventilation or hospice care.

P158 ORAL SECRETIONS SCALE – TEST-RE-TEST RELIABILITY PILOT STUDY IN A JAPANESE AMYOTROPHIC LATERAL SCLEROSIS POPULATION

NAKAYAMA Y[1], OGURA A[1], MATSUDA C[1], SHIMIZU T[1], CAZZOLLI P[2], BROOKS BR[3]

[1]*Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan,* [2]*The ALS/Neuromuscular Education Project, Ohio, United States,* [3]*Carolinas Neuromuscular/ALS-MDA Center, Charlotte, United States*

E-mail address for correspondence: nakayama-yk@igakuken.or.jp

Keywords: oral secretions scale, reliability, NIV

Background: The Oral Secretions Scale (OSS) permits identification of oral secretion burden that may limit usefulness of non-invasive positive pressure ventilation (NPPV).

Objective: Investigate the Test-Re-Test Reliability of OSS as a parameter to evaluate oral secretions in a Japanese ALS patient cohort.

Methods: In the OSS, saliva retention and swallowing are evaluated using 5 grades (normal, mild, moderate, severe, and most severe). ALS patients (5 males and 10 females) with a mean duration of disease 6.4±5.2 (standard deviation) years (range 1-16 years) participated. NPPV was employed in 5/15 patients. Dysphagia was present in 12/15 patients (9/12 had gastrostomy tube placement). An intermittent suctioning machine was applied in 6/15, and a low-pressure continuous suctioning machine in 4/15. In this study, we examined the consistency rate and inter-rater reliability (kappa coefficient) of the scores obtained by 4 nurses separately observing and rating 15 ALS patients. The study was 90% powered to detect a Kappa: 0.90 or above (almost perfect correlation).

Results: For the total 15 patient dataset Kappa was 0.66 with fixed-marginal kappa: 0.55 and free-marginal kappa: 0.57. In 4 patients scoring 4 and 2 patients scoring 0, the results of evaluation were consistent among the 4 nurses. In 1 patient scoring 4/3 and 6 patients scoring 3/2 and 2 patients scoring 2/1 or 1/0, there was only a 1 point difference among them. However, when classifying the subjects as those with a score of 2 or higher and those with 1 or lower, there was only one difference in the results of the evaluation. The subset kappa coefficient ranged from 0.64 to 0.73. The results of evaluation were less consistent when oral ingestion was possible in the presence of dysphagia, and when the grade of saliva retention/leakage depended on the posture and time.

Conclusions: OSS score reliability among 4 evaluators and 15 patients was 0.66 (substantial correlation). However, when classifying the subjects as those with a score of 2 or higher and those with 1 or lower, there was perfect correlation with only one difference in the results of evaluation among the 4 raters for the OSS score rating. These results for a 5-category scale indicate excellent inter-rater reliability of the OSS score rating among multiple independent raters.

Reference:

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P159 SUPINE VITAL CAPACITY REVISITED

RUDNICKI S[1], INGERSOLL E[2], ARCHIBALD D[2], CUDKOWICZ M[3], GRIBKOFF V[2], BOZIK M[2], THE KNS-760704-CL201 STUDY GROUP[4]

[1]*University of Arkansas Medical Center, Little Rock, AR, United States,* [2]*Knopp Neurosciences Inc., Pittsburgh, PA, United States,* [3]*Massachusetts General Hospital, Boston, MA, United States,* [4]*Multiple locations, United States*

E-mail address for correspondence: eingersoll@knoppneurosciences.com

Keywords: vital capacity, dyspnea, orthopnea

Background: Increases in the difference between percent predicted vital capacity (VC) in supine (sVC) compared to upright (uVC) position may be associated with dyspnea and orthopnea in ALS (1). As reference, the mean ΔVC (uVC minus sVC) in healthy controls is 7.5 + 5.7%; mean ΔVCs of >13.2% and >18.9%, therefore, are >1 and >2 standard deviations (SD) greater than the mean for healthy controls, respectively (2).

Objectives: To determine if epidemiologic factors are associated with a greater ΔVC, if ΔVC changes over time, and how ΔVC is related to dyspnea and orthopnea.

Methods: As part of a Phase 2 study of dexpramipexole (KNS-760704; [6R]-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine) in ALS (KNS-760704-CL201), uVC and sVC were performed at Baseline and Week 12; the ALSFRS-R was also administered. Dyspnea and orthopnea reports are based on ALSFRS-R data. Results are reported here across treatment groups.

Results: Of 102 subjects enrolled in the study, 99 had uVC and sVC data at Baseline, 94 had data at Week 12, and 91 had data at both visits (analysis population). Baseline uVC was 90.0 + 14.2% while the Baseline sVC was 83.5 + 17.4% ($P < 0.001$, paired t-test). Week 12 uVC was 79.8 + 19.7% and sVC was 75.9 + 21.6 ($P = 0.009$, paired t-test). ΔVC at Baseline was 7.0 + 8.6%, which decreased to 3.8 + 11.0% ($P = 0.007$, paired t-test) at Week 12. At Baseline, there was no relationship between the ΔVC and age, disease duration, site of disease onset, uVC, or BMI. Baseline ΔVC for 78 subjects with no dyspnea was 6.2 + 8.0% and for 13 subjects with dyspnea was 11.8 + 10.7% ($P = 0.027$). Twenty-nine subjects reported dyspnea at Week 12 and 62 subjects were dyspnea free; ΔVC for those with dyspnea was 4.9 + 11.6% and 3.4 + 10.8% for those without ($P = 0.55$, ns). At Baseline, only 3/99 subjects had orthopnea. At Week 12, 78 subjects denied orthopnea (ΔVC 3.5 + 11.3%) and 13 reported orthopnea (ΔVC 6.0 + 9.1%) ($P = 0.45$, ns). At Week 12 only 3 subjects had a ΔVC of >18.9%; 2/3 had dyspnea and 0/3 had orthopnea. A ΔVC of >13.2% was observed in 12 subjects at Week 12; dyspnea occurred in 6 of these 12 subjects and in 23/79 subjects with ΔVC <13.2% ($P = 0.267$, ns, chi square with continuity correction). Orthopnea occurred in 4/12 subjects with ΔVC >13.2% at Week 12, and in 9/79 subjects with a ΔVC <13.2% ($P = 0.115$, ns, chi square with continuity correction).

Conclusions: The relationship between ΔVC, dyspnea, and orthopnea may be less robust than previously thought. This may be in part related to the tendency of the ΔVC to lessen over time, and that some decline in VC when supine is normal.

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P160 THE BEHAVIOR OF SLEEP QUALITY IN PATIENTS WITH POST-POLIO SYNDROME ASSOCIATED WITH FATIGUE

SILVA T[1,2], OLIVEIRA A[1,2], QUADROS A[1,2], MOREIRA G[1], PRADELLA-HALLINAN M[1], TUFIK S[1]

[1]*UNIFESP - EPM, São Paulo, Brazil,* [2]*ABRELA, São Paulo, Brazil*

E-mail address for correspondence: tatimsilva@gmail.com

Keywords: post-polio syndrome, sleep, fatigue

Background: Post-polio syndrome represents new neuromuscular symptoms in patients with previous poliomyelitis. These symptoms include new weakness, fatigue, joint and muscle pain, sleep disturbances and intolerance to cold.

Objective: To analyze the behavior of sleep quality and sleep-related disordered breathing (SRDB) in patients with a history of poliomyelitis and with current post-polio syndrome (PPS) associated with fatigue.

Material and method: We prospectively studied 60 consecutive patients with PPS. All 60 patients were accompanied by our team for a 1-year period at Federal University of Sao Paulo. Our team abstracted the features of acute polio and PPS. For sleep evaluation we applied the polysomnography exam, Epworth sleepiness scale (ESS), Stanford sleepiness scale (SSS), mini-sleep questionnaire (MSQ), as well as fatigue severity scale (FSS), analogical visual scale (AVS) for pain, and Barthel scale for daily living activities.

Results: The features of PPS were fatigue, new weakness, hyper somnolence, and musculoskeletal pain. Fatigue evaluation revealed two groups: with fatigue (group F, N = 48) and without fatigue (WF, N = 12). Twenty percent of our 60 patients were using continuous positive airway pressure (CPAP) for treatment of obstructive sleep apnea (OSA). The mean of OSA was 20.2 and 15.3 per hour in groups F and WF, respectively. The mean apnea/hypopnea index (AH) was 7.1 and 5.2 per hour, periodic limb movements (PLM) index was 5.0 and 6.6 per hour in patients in these respective study groups. The overall mean age at onset of symptoms of PPS was 47 years, and the mean latent period after acute polio was 46 years. Hypersomnolence was the commonest SRDB symptom, present in 33 of the 60 patients. Pain was referred for 33% of patients in group WF, and 75% in group F. Snoring was noted in 81% of patients in group F, and 25% in group WF. The ability for activities of daily living was good in 50 of the 60 patients.

Conclusion: PPS patients with and without fatigue do not differ in quality of sleep. Therefore, bad quality of sleep seems to be a new symptom, independent of others, for patients with PPS.

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THEME 9 COGNITIVE AND PSYCHOLOGICAL ASSESSMENT AND SUPPORT

P161 THE LIVED EXPERIENCE OF FATIGUE IN MND: A QUALITATIVE STUDY FROM THE PATIENTS' PERSPECTIVE

GIBBONS C[1,2], THORNTON E[2], YOUNG C[1,2]

[1]*Walton Centre for Neurology and Neurosurgery, Liverpool, United Kingdom,* [2]*University of Liverpool, Liverpool, United Kingdom*

E-mail address for correspondence: youngca@btinternet.com

Keywords: fatigue, qualitative analysis

Objectives: The evaluation of patients' perceptions of fatigue in MND.

Methods: Fourteen patients with MND under the care of the Walton Centre for Neurology and Neurosurgery (Liverpool, U.K.) took part in semi-structured interviews. Transcripts were triangulated between the interviewer, a consultant neurologist and a health psychologist and analysed using an Interpretive Phenomenological Analysis (IPA) framework.

Results: IPA analysis revealed 7 main themes:- Physical, Mental, Emotional, Social, Progression, Activity Restriction and Rest. The experience of fatigue was conceptualised as physical, affective and to a lesser extent, cognitive. Physically, fatigue was experienced as a combination of motor failure following minimal muscle use and 'whole-body' tiredness unrelated to exertion or rest. Some patients reported difficulties concentrating, especially whilst doing tasks that involved muscle use (ie holding a book). Initiation of movement, stress, anxiety and breathing difficulties were associated with increased fatigue. Fatigue was perceived to be progressive and unremitting over the course of the illness. Increased fatigue whilst carrying out activities of daily living (ADLs) led to frustration as well as adaptive changes in social motivation, periodic rest and pre-planning of activities.

Discussion: Fatigue appears to be experienced in different ways, both as an inability to sustain motor function and as a pervasive tiredness. Fatigue had an impact on patients' day-to-day lives and the way in which they planned ADLs and social activities.

Conclusion: The results of this study support a multi-dimensional model of fatigue in MND.

P162 PREDICTING QUALITY OF LIFE OF PEOPLE WITH MND: EXPLORING THE ROLE OF PSYCHOLOGICAL DISTRESS AND FUNCTIONAL STATUS

GIBBONS C[1,2], THORNTON E[2], TENNANT A[3], EALING J[4], MITCHELL D[5], SHAW P[6], TALBOT K[7], YOUNG C[1,2]

[1]*Walton Centre for Neurology and Neurosurgery, Liverpool, United Kingdom,* [2]*University of Liverpool, Liverpool, United Kingdom,* [3]*University of Leeds, Leeds, United Kingdom,* [4]*Hope Hospital, Manchester, United Kingdom,* [5]*Royal Preston Hospital, Preston, United Kingdom,* [6]*University of Sheffield, Sheffield, United Kingdom,* [7]*University of Oxford, Oxford, United Kingdom*

E-mail address for correspondence: youngca@btinternet.com

Keywords: quality of life, depression, functional status

Objectives: To evaluate the predictive power of depression, anxiety and functional status for quality of life (QoL) in MND.

Methods: Eighty six patients from five regional MND care centres in the UK completed questionnaires either at home or during a routine clinic appointment. Anxiety and depression were measured by using the Hospital Anxiety and Depression Scale (HADS), functional status was measured using the ALSFRS-R, and patient quality of life was measured using the World Health Organization's WHOQOL-BREF measure.

Results: Hierarchical multiple regression was used to assess the ability of measures of psychological distress (anxiety and depression) and functional status to predict patient quality of life. Predictor variables were entered into the model based on Pearson's correlation values with the dependant variable. The variance explained by the model as a whole was 42.9%, $F(3,84) = 22.00$ $P<0.001$. The largest single predictor of quality of life in MND was depression (beta=0.540); neither anxiety ($t(3) = 1.67$ $P=0.10$) nor functional status ($t(3)=-1.73$, $P = 0.09$) made a significant unique contribution to the model. Depression was also the only significant predictor of item 1 of the WHOLQOL instrument "How would you rate your quality of life?"

Discussion: The results show depression to be an important variable for predicting QoL in our model. This is congruent with previous research findings in MND that depict depression as correlated to health-related QoL and suggest that physical function is not directly related to patient QoL in MND.

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P163 THE CHALLENGES OF CARE-GIVING: CONTRIBUTIONS OF COGNITIVE FUNCTION, BEHAVIOUR, AND SOCIAL COGNITION DIFFICULTIES IN MND

FISHER F

Calvary Health Care Bethlehem, Melbourne, Australia and Victoria University, Melbourne, Australia

E-mail address for correspondence: fionaf@bethlehem.org.au

Keywords: care-giving, cognition, behaviour

Background: There is a growing conceptualisation of Motor Neurone Disease (MND) as a disorder affecting multiple systems, with dysfunction in anterior cortical regions associated with mild to severe cognitive impairment and behavioural dysfunction in up to 50% of patients. The overlap of MND with Frontal Temporal Dementia (FTD) in a small percentage of cases is also now well established. The psychological impact of providing care for someone with MND who also experience changes in behaviour and cognition is not well documented. While published FTD literature reports increased psychopathology and burden amongst caregivers, it is unclear whether caregivers of persons with MND (PwMND) experience similar levels of psychological distress when cognitive and behavioural changes are present.

Objectives: The current research aimed to examine the relationship between cognitive impairment, behaviour change, social cognition, and carer burden and psychological wellbeing, in a group of PwMND and their caregivers.

Methods: Thirty-nine participants with formally diagnosed MND (46% females and 54% males; mean age 66.03 years) and their nominated caregiver (77% female, 33% male; mean age 57.20 years) were recruited through specialist MND clinics. Demographic information (eg age, months since symptom onset and MND phenotype) was collected for all participants. Level of functioning (ALSFRS-R) was rated by the treating neurologist. The study assessed cognition (Addenbrooke's Cognitive Examination - Revised), behaviour (Cambridge Behavioural Inventory, and Frontal Behavioural Inventory), and social cognition (Reading the Mind in the Eyes Test).

Results: Participants were grouped according to level of cognitive impairment. Participants with bulbar onset MND were not over-represented in the cognitively impaired group ($P>0.05$).

Those who were cognitively impaired (36%) were also significantly worse in their recognition of emotional expressions (ie reduced social cognition) than those with preserved cognitive functioning ($P<0.001$). Cognitively impaired participants were also rated as being more adynamic (ie loss of interest and motivation) by their caregivers ($P<0.05$). Observed group differences in social cognition and adynamia occurred independently of physical disability and functional impairment as measured by the ALSFRS-R.

Cognitive impairment and social cognition were not associated with increased caregiver burden or depressive and anxious symptomatology in caregivers. However, correlational analyses showed a significant positive relationship between caregiver burden and adynamia in PwMND; such that the presence of adynamia in PwMND was associated with higher levels of caregiver burden.

Conclusions: Levels of caregiver burden, as well as symptoms of anxiety and depression were not significantly associated with the presence of cognitive impairment or reduced social cognition in PwMND. Overall poor motivation and loss of interest was what carers identified as most challenging.

P164 DECISIONS ON LIFE SUSTAINING TREATMENT: THE ROLE OF COGNITIVE FACTORS

SORG S, HEIZMANN C, LUDOLPH AC, LULÉ D

Department of Neurology, University of Ulm, Ulm, Germany

E-mail address for correspondence: sonja.sorg@uni-ulm.de

Keywords: cognitive functioning, LST-decisions

Background: In recent years, ALS has been understood as a multiple system disorder that comprises cognitive impairments in addition to the loss of physical function. Evidence and opinions on the extent of cognitive impairments differ. The most salient cognitive deficits relate to executive functions like attention, memory, and verbal fluency. In most studies, bulbar onset patients seem to suffer from stronger cognitive impairments than spinal onset patients. To our knowledge, there are no data on the relationship between the status quo of cognitive functioning and decisions regarding life sustaining treatment.

Objectives: First, we wanted to investigate cognitive functioning in our patient sample; and secondly, we aimed to present data on the association between cognitive impairment and decisions regarding life sustaining treatment.

Methods: 48 patients and 73 controls were tested by a version of the d2 attention test that relied on eye movements as reaction indicators. The d2 measures vigilance (missings) and behavioural inhibition (false alarms). Speech comprehension and verbal memory/intelligence were assessed by standardised instruments. Verbal fluency was tested in patients and compared with controls (exclusion: score ≤ 3 on ALS-FRS speech item). Depressive symptoms and quality of life were assessed by standardised instruments. Decisional status was measured for NIV, PEG, and IV.

Results: Controls had significantly higher scores in cued verbal recall/verbal intelligence than both spinal and bulbar onset patients ($P = 0.000$). This held true after we controlled for level of education. Both patient groups scored lower on the d2 vigilance score, but only bulbar patients showed signs of behavioural disinhibition. In our sample, no differences were found in speech comprehension as well as verbal fluency between controls and patients. None of the cognitive variables were related to the decisional status on life sustaining treatment options and psychosocial adaptation.

Conclusion: We found no evidence for an association between cognitive impairment and decisions in favour of life sustaining treatment. This implies that patients with mild cognitive deficits may well be able to make adequate decisions on this matter.

P165 APATHY AND DEPRESSION IN ALS: TWO DIFFERENT CONSTRUCTS

CALVO A, MOGLIA C, CANOSA A, GALLO S, BERSANO E, MELISA A, TAVERNA R, MONTUSCHI A, CHIÒ A

ALS Center, Neuroscience Department, University of Turin, Turin, Italy

E-mail address for correspondence: andreacalvo@hotmail.com

Keywords: apathy, depression

Background: Apathy is a frequent symptom in ALS patients, possibly reflecting a dysfunction of frontal lobes and of the frontal-subcortical circuits. Apathy is a multidimensional concept referring to a mental state of reduced interest and

participation in various activities, characterized by a sense of indifference which is not present in depression, where mood is distinctly negative and causes emotional suffering. In Parkinson's disease the absence of a correlation between apathy and depression has been demonstrated. However, there are no studies on the relationship between apathy and depression in ALS.

Aim: To evaluate the presence and the correlates of apathy and depression in patients with ALS.

Methods: Forty one patients with ALS consecutively seen in our ALS Clinic between 1 January 2010 and 28 February 2010 were administered with the Lille Apathy Rating Scale (LARS), a patient-based assessment of apathy in PD, the Hospital Anxiety and Depression Scale (HADS), as a brief instrument to measure psychological distress, the McGill Quality of Life Questionnaire (MQOL) and the Single Item Scale (SIS), rating overall quality of life. The functional status of patients was evaluated with ALSFRS-R.

Results: The patients (21 men and 20 women; 31 spinal and 10 bulbar onset) had a mean age of 65.5 (SD 11.1) years. Their mean ALSFRS-R score was 36.8 (range 19–47). According to the rating scales cut-off scores, mild to severe depression was present in only 4 (9.8%) patients, anxiety in 14 (34.1%) patients and apathy in 13 (31.7%) of patients. Apathy scores were not related to depression (P = 0.07) or anxiety (P = 0.85) scores. However, among the four domains of the LARS, action initiation (P = 0.01) and intellectual curiosity (P = 0.03) were related to HADS depression scores, while emotion (P = 0.91) and self-awareness (P = 0.46) were not related. LARS scores were not correlated with MQOL scores. Conversely, in multivariable analysis, both depression and anxiety scores showed a strong correlation with quality of life scores.

Conclusions: Depression and apathy are different constructs in ALS. Apathy was detected in about one third of ALS cases in our series, in keeping with the description of frontotemporal features in 20 to 50% of ALS patients. Apathy does not seem to worsen patients' QOL, differently from its negative impact on caregivers' QOL.

P166 A STUDY OF ALEXITHYMIA AND DEPENDENCE ON EXERCISE IN ALS

ROY-BELLINA S[1], LECORNEY A[2], CAMU W[1], PAGEOT N[1], JUNTAS-MORALES R[1], GÉLY-NARGEOT M-C[2]

[1]*Clinique du Motoneurone, Montpellier, France,* [2]*Université Paul Valéry, Montpellier, France*

E-mail address for correspondence: s-roy@chu-montpellier.fr

Keywords: alexithymia, exercise

Background: Different authors agree about the 'kindness' of ALS patients. Aside from this surprising characteristic, some others write about 'the apparent indifference' of the patients when facing this devastating disorder.

Objectives: Taking into account that addictive behaviors are associated with an alexithymic profile on one hand, and on the other hand that ALS patients often practice sport intensively, we searched for a link in ALS patients between 'addiction' to exercise, and alexithymia.

Methods: Sixteen ALS patients (8 men, 8 women, aged 63 yrs±8.3), with a mean duration of disease of 37 months (±34) and a mean ALSFRS score of 37 (±4.77), and 16 controls (7 men, 9 women, aged 60 yrs±9.37) were evaluated with the following: MMSE, EDF (frontal scale), BDI-II and MADRS (depression), STAI-Y (anxiety), Ekman test (identification of emotional states from 60 facial expressions), Emotional State Questionnaire (ESQ), Bermond Vorst Alexithymia Questionnaire (BVAQ) and Exercise Dependence Scale-Revised (EDS-R). Patients with dementia were excluded from this study.

Results: ALS patients were significantly more depressed (P = 0.009) and anxious (P = 0.03) than controls. There were no differences regarding dependence on exercise and alexithymia. A trend was noted for a difference in the ability to identify emotions in the Ekman test (P = 0.06). We could not find any correlation between our tests (dependence to exercise, depression, anxiety, emotional profile) and alexithymia : EDS-R*BVAQ (P = 0.45), BVAQ*BDI-II (P = 0.3), BVAQ*MADRS (P = 0.21), BVAQ*STAY-E (P = 0.1), BVAQ*STAY-T (P = 0.24), BVAQ*ESQ (P = 0.29).

Discussion and conclusions: Conversely with several studies we found that ALS patients were more depressed than controls, and also more anxious. No specific anxious profile could be determined from our analysis. Our hypothesis of a link between 'addiction' to exercise and alexithymia could not be confirmed.

P167 BEHAVIOURAL CHANGES IN MOTOR NEURON DISEASE

LILLO P, MIOSHI E, HODGES JR

Prince of Wales Medical Research Institute, University of New South Wales, Sydney, NSW, Australia

E-mail address for correspondence: p.lillo@powmri.edu.au

Keywords: behavioural changes, carer burden, apathy

Background: Motor Neuron Disease (MND) has traditionally been considered a pure motor syndrome which spares aspects of cognition and behaviour, although in recent years it has been suggested that up to 50% of patients with MND may develop frontal dysfunction which in some cases is severe enough to reach criteria for Frontotemporal Dementia (FTD). Behavioural changes, emotional lability and impaired social cognition are part of the clinical spectrum but estimates of the frequency of presentation remain unknown.

Objectives: To assess the frequency of behavioural changes in patients with MND, to assess the potential impact of behavioural changes on carer burden and to compare the behavioural changes in MND vs. behavioural variant frontotemporal dementia (bvFTD).

Methods: A postal survey was completed by patients with MND (n = 92) and their carers (n = 81) from New South Wales. The survey covered motor function and neuropsychiatric symptoms in the patients. Carers received questionnaires regarding their current mood and burden of care. Carer reports were compared with carers of patients with bvFTD (n = 25).

Results and discussion: Carers reported moderate-severe behavioural changes in MND patients involving principally motivation (40%). A substantial proportion of MND patients manifested behavioural changes of the type seen in FTD with 11% fulfilling criteria of frontotemporal dementia. Less than 25% of the carers reported depression but 44% reported severe burden. The carer burden was explained predominantly by the presence of abnormal behaviour. As expected, behavioural changes were significantly more frequent in bvFTD than in MND patients (P < 0.001).

P168 DEFICITS IN CONCEPT FORMATION IN AMYOTROPHIC LATERAL SCLEROSIS

ELMAN L[1], LIBON D[2], GUNAWARDENA D[1], MCMILLAN C[1], AVANTS B[1], MCCLUSKEY L[1], GROSSMAN M[1]

[1]*University of Pennsylvania Medical Center, Philadelphia, PA, United States,* [2]*Drexel University College of Medicine, Philadelphia, PA, United States*

E-mail address for correspondence: elmanl@uphs.upenn.edu

Keywords: frontotemporal lobar degeneration, executive function, structural MRI

Background: The type of cognitive impairment that is most commonly seen in patients with amyotrophic lateral sclerosis (ALS) is in the spectrum of frontotemporal lobar degeneration (FTLD), which includes a prominent dysexecutive component. Letter and category fluency tasks are the most commonly used measures to screen for dysexecutive function but are not ideal in this population because they are timed and include a motor component.

Objectives: We chose an untimed measure of concept formation for administration to ALS patients to determine if this measure would discriminate between ALS patients who are cognitively intact (ALS-nl), have mild cognitive impairment (ALS-CI), or have FTLD (ALS-FTLD). We related performance on the concept formation task to MRI assessments of cortical thickness.

Methods: A standard screening procedure divided patients into three groups, ALS-nl, ALS-CI, ALS-FTLD. They performed the Delis-Kaplan Executive Function System Sorting Test (DKST). This involves sorting 16 stimulus words printed in various fonts and styles on differently shaped cards. A subset of 16 patients underwent high resolution structural MRI scans.

Results: ALS-CI and ALS-FTLD groups had significantly lower age-corrected scaled scores compared to the ALS-nl group on measures of free, recognition and composite free/recognition sorting. The ALS-FTLD group scored lower than the ALS-CI group on all sorting measures. Regression analyses related measures of action naming, single word semantic knowledge, and mental search/working memory to sorting performance. MRI showed cortical thinning in bilateral premotor/dorsolateral prefrontal regions and left anterolateral temporal cortex. Reduced performance on the composite measure of free/recognition sorting was related to cortical thinning of left dorsolateral prefrontal cortex.

Discussion and conclusions: ALS is a multi-system neurodegenerative disorder. Cognitively impaired or demented ALS patients performed poorly on untimed concept formation tasks that do not depend on motor functioning. Disease in prefrontal regions interrupts a network of cognitive operations leading to the concept formation deficit in ALS.

P169 IMPAIRED INSIGHT FOR COGNITIVE DECLINE IN ALS WITH COGNITIVE IMPAIRMENT

FLAHERTY-CRAIG C, BROTHERS A, SVOBODA R, SIMMONS Z

Penn State College of Medicine/Hershey Medical Center, Hershey, PA, United States

E-mail address for correspondence: abrothers@hmc.psu.edu

Keywords: cognitive impairment, insight, anosognosia

Background: It is now generally accepted that amyotrophic lateral sclerosis (ALS) is a complex multisystem disorder which is accompanied by symptoms of frontotemporal lobar degeneration (FTLD) in up to half of patients. Loss of insight, a characteristic deficit of FTLD, has been reported in patients with ALS with regard to behavior, but loss of insight for cognitive deficits (cognitive anosognosia) has received less attention.

Objectives: To examine cognitive anosognosia in patients with ALS and cognitive impairment (ALS-Ci).

Methods: Participants included ALS subjects with one or more deficiencies on brief exam measures of letter fluency, COGNISTAT abstractions, or COGNISTAT judgment (ALS-Ci), ALS subjects with intact cognitive brief exams (ALS control), and healthy controls. Using Barrett *et al's* method, cognitive anosognosia ratios were calculated for four motor-free neuropsychological assessments, including verbal problem-solving (Guilford Alternate Uses), non-verbal problem-solving (Guilford Missing Cartoons and Expression Grouping), and emotional recognition (Ekman Faces). Descriptive statistics are reported for demographic and disease data. One-way Analysis of Variance was used to compare test performance and anosognosia ratings among all three groups. Post-hoc analyses were conducted using the Games-Howell test.

Results: There were 56 subjects: 25 ALS-Ci, 13 ALS controls, and 18 healthy controls. *Participant Characteristics*: No statistical difference was found between groups based on demographic variables or disease characteristics. On average, participants were 62.4 with 13.7 years of education. Patients had an average forced vital capacity (FVC) of 67.7, with 35.4 months since onset. *Test Performance:* ALS-Ci participants performed worse on Alternate Uses compared with ALS-control ($P = 0.003$) and healthy control ($P < 0.001$) participants. ALS-Ci participants fared worse than healthy controls on Missing Cartoons ($P = 0.002$) and Expression grouping ($P < 0.001$). On Ekman Faces, the ALS-Ci group performed significantly worse than both the ALS-control group ($P = 0.02$) and the healthy control group ($P = 0.002$). *Insight*: ALS-Ci participants overestimated their performance on the Alternate Uses task compared to healthy controls for both the pre-test ($P = 0.03$) and post-test ($P = 0.001$). ALS-Ci participants over-estimated their performance compared to healthy controls on Missing Cartoons post-test ($P = 0.04$). A significant difference was found between ALS controls and healthy controls on the Missing Cartoons pre-test ($P=0.04$). No differences were found for anosognosia ratios for Expression Grouping or Ekman Faces.

Discussion and conclusions: Not only did ALS-Ci patients perform significantly worse than controls on each task, they also tended to over-rate their performance on measures of verbal and non-verbal problem solving alike. The ALS control group over-rated their performance compared to healthy control subjects. Further studies are needed to address whether minimization of cognitive deficits in ALS represents a psychological response to the cognitive decline by the patient with co-existing motor neuron disease, or a genuine indication of loss of insight. Areas of impact include treatment compliance and safety, affecting longevity and quality of life.

P170 THE CLINICAL UTILITY OF PRIMITIVE REFLEX EXAMINATION IN MOTOR NEURON DISEASE

FISHER F[1], REARDON K[1], PAVLIS A[2]

[1]*Calvary Health Care Bethlehem, Melbourne, Australia,* [2]*Victoria University, Melbourne, Australia*

E-mail address for correspondence: fionaf@bethlehem.org.au

Keywords: primitive reflexes, cognition

Background: MND is no longer regarded as primarily a motor system disease. Research has consistently identified mild to severe cognitive impairment and behaviour disturbances in as many as 50% of individuals with MND. Accurate detection of cognitive impairment is essential as those with coexisting frontotemporal syndrome have reduced life expectancy and will require additional support with decision-making, particularly in regards to life-prolonging interventions.

The reappearance of primitive reflexes in adulthood has traditionally been taken to be an indicator of pathological brain functioning, and likely indicative of a 'release phenomenon' when loss of inhibition occurs in neural networks. The re-emergence of primitive reflexes has also been described as a clinical indicator of prefrontal dysfunction; however evidence for a causal relationship is inconsistent. Primitive reflexes have been suggested to be useful in assessing the severity of Parkinson's disease, and also in distinguishing Parkinson's disease from vascular parkinsonism. While primitive reflexes are commonly assessed during routine neurological examination, the presence of primitive reflexes in individuals with MND, and the potential clinical utility of primitive reflex examination has not been extensively examined.

Objectives: The current research aimed to examine the relationship between the presence of primitive reflexes and the presence of significant cognitive and behavioural impairment in persons with MND.

Methods: The sample comprised 46 participants (42% females and 48% males) with formally diagnosed MND recruited through specialist MND clinics. Demographic information (eg age, months since symptom onset, and MND phenotype) was collected for all participants. Examination of primitive reflexes and current functioning (ALSFRS-R) was conducted by neurologists during routine neurological examinations. Cognition, behaviour, and social cognition were assessed using the Addenbrooke's Cognitive Examination-Revised (ACE-R), the Frontal Behavioural Inventory (FBI), and the Reading the Mind in the Eyes Test (Eyes Test) respectively.

Results: Sixteen ALS patients (34.8%) had at least 1 positive primitive reflex, while 13 participants (28.3%) had 2 or more positive primitive reflexes. Chi-squared analyses revealed that the presence of 1 or more positive primitive reflexes was not significantly associated with age, gender, disease duration, or MND phenotype ($P > 0.05$). Furthermore, Chi-squared tests found no significant association between the re-emergence of primitive reflexes and the presence of cognitive impairment, behavioural disturbance, and social cognition ($P > 0.05$).

Conclusions: While positive primitive reflexes were present in 34.8% of the sample, their utility as an additional clinical marker of cognitive dysfunction was not supported. Thus, the examination of primitive reflexes during routine neurological examination does not appear to assist recognition of cognitive impairment in MND.

P171 DEVELOPMENT OF A HANDS-FREE, EYE-TRACKING VERSION OF THE TRAIL MAKING TEST

SHARMA R, HICKS S, KHAN A, BERNA C, KENNARD C, TALBOT K, TURNER M

Oxford University, Oxford, United Kingdom

E-mail address for correspondence: martin.turner@clneuro.ox.ac.uk

Keywords: eye-tracking, cognitive, frontotemporal

Background: The Trail Making Test (TMT) is a two-part written tool for detecting executive dysfunction. Part A consists of joining a 'trail' of ascending numbers, which serves as a control for a second more challenging Part B where the 'trail' alternates between ascending numbers and alphabetical letters. Executive dysfunction is common in patients with ALS. Both neuropsychological assessment and oculomotor functions can help inform the wider cerebral disease process in ALS (1). However many neuropsychological tests may be impossible in the presence of upper limb weakness or speech impairment.

Objectives: To design a hands-free version of the TMT performed visually using eye-tracking equipment, and to assess its reliability compared with the standard written version.

Methods: Forty healthy volunteers were recruited. A head-mounted Eyelink® eye-tracking device was used in conjunction with a visual display of the trails. To eliminate any immediate practice effects there was an interval of one week between testing each version of the TMT. The first twenty volunteers performed the visual followed by the written version one week later, and the remaining twenty vice versa. The B:A ratio of time in seconds for each task was used as a standardised reliability outcome measure across the two different paradigms.

Results: Good correlation (r2 = 0.6) was found between written and visual TMT outcome measures.

Discussion: The TMT transferred well to the eye-tracking environment and is independent of limb or speech impairments. The oculomotor TMT provided additional information about the errors made and the search strategies used by subjects, which could not be derived from the traditional written version.

Conclusions: Eye-tracking has significant potential as a tool to explore cognitive impairment as well as specific oculomotor abnormalities. It has subsequently proved easily applicable to ALS patients across a range of disability in an on-going longitudinal study.

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P172 A NEW METHOD OF CATEGORISING NEUROPSYCHOLOGICAL DEFICITS IN PEOPLE WITH ALS

JORDAN N[3], PENDER N[1], PHUKAN J[2], ABRAHAMS S[4], ELAMIN M[1], BEDE P[1], HARDIMAN O[1,2]

[1]*Beaumont Hospital, Dublin, Ireland,* [2]*Trinity College Dublin, Dublin, Ireland,* [3]*Royal College of Surgeons in Ireland, Dublin, Ireland,* [4]*Department of Psychology, Institute of Psychiatry, King's College London, London, United Kingdom*

E-mail address for correspondence: orla@hardiman.net

Keywords: consensus criteria, cognitive/behaviour impairment, methodology

We present the first methodological analysis of the consensus criteria (1) in a population-based study of ALS, and we make our own recommendations for alternative analysis. The current paper is based on the initial dataset gathered by one of the authors as a part of a longitudinal Irish population-based study of ALS; the largest of its kind. Analysis of the results of an extensive neuropsychological battery (including tests of executive function, memory, language and visuospatial ability) from 51 patients with ALS, but with no co-morbid dementia, was carried out.

Results indicate that use of the Strong *et al.* (1) criteria identified 33% of patients with ALS as ALSci (ALS cognitive impairment; defined as below the fifth percentile on two or more tests of executive function). Our recommended criteria identified 13.7% of patients with ALS as having executive dysfunction and a further 31.4% as having some other cognitive impairment (memory, multidomain, language or visuospatial). Therefore, based on our analysis, 45.1% of people with ALS had some form of cognitive impairment.

Patients were categorized separately for behavioral impairment. Results of an analysis of the Frontal Systems Behaviour Scale (FrSBe) indicated that 23% of the 51 participants with ALS had behavioral impairment. In an attempt to avoid over-diagnosis of behavioural impairments, a classification of behavioural impairment is only given if the person scores more than 65 on the T-scores of 3 or more of the after subscales, unless the person's before score on the same subscale was also over 65, thus demonstrating no change over time.

Based on the results of the analysis, it appears that the Strong criteria may underestimate the number of people with cognitive impairment and the nature of impairment. The results of analysis using the Strong criteria suggests that 33% of bulbar and spinal onset participants combined were classified as cognitively impaired (ALSci), compared with 4% of the control participants. Our analysis demonstrated that in fact up to 45% of ALS participants may be impaired on one or more cognitive domains of interest. However, in our suggested analysis, a smaller percentage of 13.7% of the participants with ALS who have not been classified with FTLD had executive dysfunction (5.9% executive dysfunction, 7.8% multidomain with executive dysfunction).

In addition, a large number had impairment in other areas, such as 7.8% with memory impairment, 11.8% with multidomain impairment without executive dysfunction and 9.8% with language impairment. In addition, based on analysis using our suggested methodology, 2.6% of controls met criteria for executive impairment, in contrast with the Strong criteria, which found that 12% of controls had executive impairment, a number that seems high for a control population.

Reference:

1. Strong MJ, Grace GM, Freedman M, *et al*. Amyotrophic Lateral Scler. Jun;10(3):131–46.

P173 COGNITIVE PROFILE OF ALS PATIENTS WITH COMPARISON OF INCIDENT VERSUS PREVALENT POPULATIONS: A DOMAIN-BASED APPROACH

ELAMIN M[1,2], PHUKAN J[1,2], BEDE P[1,2], JORDAN N[1], PENDER N[1], HARDIMAN O[1,2]

[1]*Beaumont Hospital, Dublin, Ireland,* [2]*Trinity College Institute of Neuroscience, Dublin, Ireland*

E-mail address for correspondence: marwaelamin08@gmail.com

Keywords: cognitive, classification, incident

Background: Estimates of cognitive impairment in patients with amyotrophic lateral sclerosis (ALS) vary widely. Studies published to date are small, clinic based and are heavily reliant on the prevalent ALS population. The literature is further confounded by the currently used criteria (1) for cognitive impairment in ALS which focuses on executive dysfunction, when there is increasing evidence of involvement of other cognitive domains in ALS.

Objectives: 1) To describe the incidence of cognitive impairment in a population-based ALS cohort using new criteria that focus on single or multiple cognitive domain dysfunction; 2) To compare frequency of cognitive impairment in the incident ALS patients to the prevalent ALS patients.

Methods: All cases of possible, probable or definite ALS in the Republic of Ireland have been invited to participate. Demographic, clinical and neuropsychological data are collected during home visits carried out at baseline and then repeated at six monthly intervals.

Cognitive impairment in ALS patients is defined according to performance on neuropsychological tests covering five cognitive domains: attention/executive function, memory, language, visuospatial skills. Patients were then classified depending on two factors: 1) the number of cognitive domains involved (none, single or multiple); 2) the presence or absence of executive dysfunction. The frequency of each cognitive syndrome in the ALS population was compared to that in age, sex and education-matched healthy controls.

Results: To date, 167 patients have been recruited to the study. Mean age was 63.5 years, 60% were males and 33.6% of patients had bulbar-onset ALS. Incident cases comprised 83.3% of the cohort.

Twenty two patients (13.6%) fulfilled the Neary criteria for frontotemporal dementia (FTD). Compared to healthy controls, non-demented ALS patients had significantly higher frequency of single or multi-domain cognitive impairment with executive dysfunction (29.9% versus 2.6%, $P < 0.0001$). There was no significant difference between the 2 groups in frequency of single or multi-domain cognitive impairment without executive dysfunction (22.2% versus 16.5%, $P = 0.311$).

Incident ALS cases had a higher frequency of cognitive impairment compared to prevalent cases. The most striking difference was observed in the frequency of single domain executive dysfunction but it did not reach statistical significance (11.7% versus 3.7%, $P = 0.39$).

The striking absence of executive dysfunction in the prevalent cases compared to the incident cases led to an investigation into the effect of executive dysfunction on patient survival. The presence of executive dysfunction was found to be significantly associated with shorter survival in ALS patients (31 months versus 38 months, $P = 0.005$) even after correcting for multiple factors including age, bulbar onset and disease severity.

Discussion and conclusion: The incidence of FTD in a population based predominantly incident ALS cohort was 13.6%. Single and multi-domain executive dysfunction was significantly more frequent in the ALS population compared to healthy controls. The incident population had a higher incidence of impairment in all cognitive domains, in particular executive function. The fact that executive dysfunction was predominantly a feature of incident, rather than prevalent ALS patients, is likely to be secondary to its association with poorer prognosis.

Reference:

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THEME 10 IMPROVING DIAGNOSIS, PROGNOSIS AND DISEASE PROGRESSION

P174 PATIENT CARE COORDINATION AT CAROLINAS NEUROMUSCULAR/ALS – MDA CENTER: DISEASE-SPECIFIC CARE CERTIFICATION

BLYTHE A[1], WILLIAMS NM[1], BLACK KJ[1], NICHOLS MS[1], WRIGHT KA[1], BRAVVER E [1,2], DESAI U[1], BROOKS BR[1]

[1]*Carolinas Medical Center - Department of Neurology, Charlotte, NC, United States,* [2]*University of North Carolina - Chapel Hill School of Medicine Department of Neurology, Chapell Hill, NC, United States,* [3]*University of North Carolina - Charlotte Department of Kinesiology, Charlotte, NC, United States*

E-mail address for correspondence: anne.blythe@carolinashealthcare.org

Keywords: The Joint Commission, performance meaures, outcome measures

Background: DSC certification by The Joint Commission (TJC) has been designed to recognize disease management programs for superior quality. A fore-runner of these programs in neurology has been the Primary Stroke Center Certification developed in collaboration with the American Stroke Association which depends on a Stroke Core Measure Set and Stroke Performance Measure Requirements based on the Brain Attack Coalition's guidelines. Longitudinal analysis of stroke diagnosis, treatment and outcome is the key determinant of certification and re-certification. DSC Certification is currently a voluntary process. At the heart of DSC certification is a formal review of patient care data according to established guidelines. In the past, many ALS clinics participated voluntarily in ALS CARE which was a paper-based documentation of patient care encounters longitudinally that permitted assessment of each ALS Clinic's adherence to the 1999 American Academy of Neurology (AAN) ALS Practice Parameter (1) and benchmarks for ALS care provided in the real world (2, 3). In parallel, the ALS Association Center[SM] Program voluntarily certified ALS Centers based on the facilities and personnel available but did not require a longitudinal analysis of patient outcomes. The recently released expanded 2009 AAN ALS Practice Parameter (4) will be the basis for developing ALS DSC certification with TJC together with the ALS Research Group (ALSRG) in collaboration with the AAN.

Objective: Implement the first three core performance measures of the ALS DSC certification profile and collect quantitative patient data for outcomes assessment in a large ALS Clinic in the Department of Neurology situated in the third largest public healthcare system in the United States - Carolinas Healthcare System.

Methods: Ambulatory care nurse meetings with allied health staff and professional staff reviewed standardized performance measures and identified cognitive, psychiatric, and falls performance measures based on validated clinimetric scales which were implemented in intake, follow-up, and multidisciplinary clinics.

Results: Consensus regarding cognitive, psychiatric and falls performance measures required implementation of several meetings to review validation parameters and integrate the clinimetric scales with the electronic medical record. Collecting performance measures in each patient increased clinical encounter time by 8%. Collating performance measure outcomes in each patient required additional administrative time measured at 1-2% per patient for three performance measures.

Conclusions: Initiating three performance measures in an ALS clinic requires increased encounter time and increased administrative time. Increased complexity of the performance measures, coupled with increased analysis in real time of the outcomes identified by these performance measures has led us to identify increased time needs for ALS DSC certification. Implementation of additional performance measures will require attention to increased efficiencies at the clinic level.

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P175 COGNITION – AFFECT – BULBAR – RESPIRATORY – UPPER AND – LOWER EXTREMITY CLINICAL STAGING IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: CROSS-SECTIONAL ANALYSIS AT FIRST CLINIC VISIT IN 264 PATIENT VALIDATION COHORT

BROOKS BR[1,4], SANJAK MS[1,5], BRAVVER E[1,4], BOCKENEK WL[2,6], DESAI U[1,5], LINDBLOM SS[3], PACCICO T[6], WARD AL[1], DUFFY K[1], WRIGHT AK[1], WILLIAMS NM[1], NICHOLS MS[1], BLACK KJ[1], O'NEILL M[1], LANGFORD V[1,5], WALGREN K[1], RUSSO P[1], BLYTHE A[1]

[1]*Carolinas Medical Center - Department of Neurology, Charlotte, NC, United States,* [2]*Carolinas Rehabilitation - Department of Physical Medicine, Charlotte, NC, United States,* [3]*Carolinas Medical Center - Department of Internal Medicine, Charlotte, NC, United States,* [4]*University of North Carolina - Chapel Hill - Department of Neurology, Chapel Hill, NC, United States,* [5]*University of North Carolina - Charlotte - Department of Kinesiology, Charlotte, NC, United States,* [6]*University of North Carolina - Chapel Hill - Department of Physical Medicine and Rehabilitation, Chapel HIll, NC, United States*

E-mail address for correspondence: benjamin.brooks@carolinas healthcare.org

Keywords: disease specific certification, patient outcomes, performance measures

Background: Advances in ALS classification have depended upon El Escorial, Airlie House and Awaji criteria for the diagnosis. However, the need is increasing for a comprehensive assessment of disease involvement (staging) and disease severity (grading) in each patient to define the appropriate disease endo-phenotype. Early attempts at staging (1, 2, 3) were not sufficiently comprehensive nor based on established criteria. There is a need for ALS disease staging which will provide a format for ALS Disease Specific Certification by the Joint Commision based on assessment of performance measurements at individual ALS Clinics. ALS specific treatments have been developed for different features of the disease and need to be implemented at the specific stage.

Objective: Evaluate an ALS disease staging system that organizes disease-associated anatomical or domain (behavior, function) involvement together with progression milestones to provide a validated description of the extent of ALS involvement so that results of treatments may be compared and understood.

Methods: Domain definition was based on literature cross-sectional samples of 40-2456 patients at different stages and previously presented (4). The development cohort consisted of 264 unselected ALS patients from a large MDA/ALS Center who were analyzed cross-sectionally and prospectively to validate six (6) staging domains. Stage 0 - normal function; Stage - 1 no treatment; Stage 2 - no or minimal treatment; Stage 3 and 4 - treatment; Stage 5 - severe disease; Stage 6 - Hospice eligible.

Results: At the first clinic visit ALS patients above stage 0 or 1 were - Cognitive (C)-impaired (15%), behavior-impaired (4%), -mixed (2%); - Affect(A)-pseudobulbar (10%), -depressed (13%), -mixed (9%); - Bulbar(B)-speech-impaired (5%), swallow-impaired (14%), -mixed (25%); - Respiratory (R) –impaired (30%), -assisted (9%); - Upper Extremity (UE)-impaired (31%), -dependent (14%), -mixed (6%) and - Lower Extremity(LE)-impaired (19%), -dependent (21%). C-patients (16.7%) and A-patients (26.5%) were spread throughout B, R , UE and LE domains. B-patients (18.9%) and R-patients (28.7%) free of C- and A-involvement were distributed through UE and LE domains. The remaining UE- and LE-patients had no C-, A-, B- and R-involvement. Progression from initial stages through later stages proceeded over 2-4 years.

Conclusions: ALS staging permits definition of disease involvement over 6 domains on a per patient basis. Further validation is proceeding with a second validation cohort to confirm this staging system.

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P176 INITIAL DESIGN AND EVALUATION OF A NEW CLINICAL ASSESSMENT TOOL TO QUANTIFY MOTOR DEFICITS IN ALS PATIENTS: THE MADRID QUANTITATIVE NEUROMUSCULAR ASSESSMENT, MAQUINA

MORA JS, VALERA F, MACÍAS AI, HURTADO F, MARÍN S, MINAYA FJ, MASCÍAS J, CHAVERRI D

ALS Unit, Hospital Carlos III, Madrid, Spain

E-mail address for correspondence: jmora.hciii@salud.madrid.org

Keywords: clinical-assessment, timed-tests, impairment-quantification

Background: In clinical practice and trials different scales (e.g. ALSFRS-R) or tests (e.g. QMA) are used to evaluate ALS motor deficits. None is fully satisfactory. Waiting for the adequate biomarker, a permanent search for new clinical assessment tools is warranted.

Objectives: The final objective is to develop and validate a new clinical ALS assessment tool, MAQUINA (Madrid Quantitative Neuromuscular Assessment). The first step was to identify or design the most adequate tests to study, and then to evaluate them, in order to design a protocol of a limited number of easy use tests, with few technical drawbacks, prolonged applicability, and acceptable time duration.

Methods: A series of stages were followed: 1) Literature Search. Most important medical databases were searched and reviewed for published tests. A Focal Group of experienced ALS neurologists and physiotherapists selected or designed best tests for initial study based on best evidence and clinical experience; 2) An evaluation of those tests was done in up to 35 ALS patients by those professionals. Quantitative evaluation included number and percentage of technical drawbacks and tests failures, and time spent. Qualitative evaluation labeled each test 0 to 3 for facility of use, technical drawbacks, duplicity, prolonged applicability and time consuming. Best rated non duplicated tests were finally selected to compose MAQUINA.

Results: Timed bulbar tests pataka (10-repetition) and count (up to 10-count), and arm tests arm pedalling (10-rounds) and marbles (10-marbles move) were of easy use and moderate technical drawback or failure in advanced disease. Pegs was rejected for duplicity and lower reliability. Three up&go leg test variants had moderately high technical drawbacks and test failures, the up&3m walk test being best rated and so selected. Similar ratings were obtained leg pedalling

(10-rounds). Palm tapping and sole tapping (10-repetition) had higher technical drawback or failure in advanced disease. Technical adjustments in legs tests were made to extend time of use. Total performance time of finally selected tests is 20 minutes.

Conclusions: Based on best evidence and clinical experience, eleven timed tests, three in right and left sides, were preselected and evaluated. Eight were selected based on least technical drawbacks, duplicity or time consumed and prolonged applicability. Reliability studies are described in another communication. Comparison with current quantified muscle assessment tests and functional scales and validation of the MAQUINA protocol is under way.

Acknowledgment: Work supported by Fundación Mutua Madrileña.

P177 INTER- AND INTRA-RATER RELIABILITY OF DESIGNED TESTS TO ASSESS ALS PATIENTS WITHIN THE MADRID QUANTITATIVE NEUROMUSCULAR ASSESSMENT, MAQUINA

MORA JS, MACÍAS AI, VALERA HF, HURTADO F, MARIN S, HERNÁNDEZ M, MORAN Y, SALAS T

ALS Unit, Hospital Carlos III, Madrid, Madrid, Spain

E-mail address for correspondence: jmora.hciii@salud.madrid.org

Keywords: clinical-assessment, timed-tests, impairment-quantification

Background: A battery of timed tests to quantify motor deficits in ALS patients is being designed. Quantitative and qualitative evaluation of facility of use, technical drawbacks and time applicability was done in preselected tests for bulbar, arms and legs impairment. Reliability, validation and comparison studies are necessary to consider this battery a useful tool for clinical practice and trials.

Objectives: To analyze inter- and intra-rater reliability of eleven initially preselected timed test to determine best tests for inclusion in MAQUINA.

Methods: Up to thirty-five ALS patients were examined by two physiotherapists trained in the standardized tests protocol. The patients were examined twice by each examiner in random order and established times. The exam included 2 timed tests of bulbar impairment, 4 of arms and 5 of legs impairment, three of them in right and left sides. Five standardized tests to quantify muscle strength in selected arm and in leg muscle groups, and 1 test of respiratory function were also analyzed, for further comparison studies. The Kappa index was used to determine inter-rater reliability, with 95% CI. Statistical analyses to establish absolute and relative proportions were carried out with SPSS 15.0 for Windows. Reliability analysis was obtained using an index of acceptable Kappa (0.6–0.8).

Results: Bulbar impairment tests, pataka and count, had very good inter- and intra-rater agreements, (Kappa 0.8–1.00). Arms impairment tests palm tapping and pedaling had very good inter- and intra-rater agreements, marbles had very good intra-rater and good inter-rater (Kappa 0.6–0.8) agreements, and pegs had moderate (Kappa 0.4–0.6) intra-rater and fair (Kappa 0.2–0.4) inter-rater agreements. Legs impairment tests sole tapping, pedaling, and up&3m walk had very good inter- and intra-rater agreements, up&1m walk and up&go had very good intra-rater and good inter-rater agreements. Slow vital capacity measurements had very good intra- and inter-rater agreements. Quantified knee flexion and extension and grip strength had very good intra- and inter-rater agreements, elbow flexion and extension had good intra- and inter-rater agreements. No significant differences were observed between dominant and non-dominant limb.

Conclusions: 1) The intra- and inter-rater reliability was good to very good (Kappa above 0.6) in all tests preselected for MAQUINA, except in pegs for arm impairment and so it was excluded; 2) Comparison studies with most frequently used ALS clinical assessment tools, ALSFRS-r and QMA quantified muscle strength, to validate a protocol for MAQUINA, are under way.

Acknowledgment: Work supported by Fundación Mutua Madrileña.

P178 SIT TO STAND AND STAIR CLIMBING ARE MORE SENSITIVE THAN 25 FOOT WALK TEST AND TIMED UP AND GO IN PREDICTING PROGRESSION IN AMBULATORY AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

SANJAK M[1,2], GANDHI RB[1], RUSSO P[1], DESAI U[1,3], BRAVVER E[1,3], BROOKS BR[1,3]

[1]*Carolinas Medical Center Department of Neurology, Charlotte, NC, United States,* [2]*Department of Kinesiology, University of North Carolinas, Charlotte, NC, United States,* [3]*Department of Neurology, University of North Carolina, Chapel Hill, NC, United States*

E-mail address for correspondence: Mohammed.Sanjak@carolinas healthcare.org

Keywords: outcome measure, sit to stand test, stair climbing test

Background: Evaluation of functional motor impairments in ALS patient is necessary for proper clinical decision making, natural history, and efficacy of therapeutics. ALSFRS-R and forced vital capacity (FVC) are among the most used tools for this purpose. Timed functional tests such as sit to stand (STS), stair climbing (SC), 25 foot walk test (25FWT) and time up and go (TUG) are quantitative, reliable, and easy to perform and frequently used in other neurodegenerative diseases but they are less commonly applied in ALS.

Objective: To compare the rate of progression in ALSFRS-R total and its sub scores of walking, stair, and respiratory function with the rate of decline in STS, SC, 25FWT, and TUG in 16 ambulatory ALS patients.

Methods: Sixteen ambulatory patients were evaluated by ALSFRS-R, FVC, and timed mobility tests consisting of STS, SC, 25FWT, at baseline, and were followed every 3 months up to 9 months. ALSFRS-R and FVC, 25FWT and TUG were evaluated using standard procedures. STS was evaluated as the time it took the patient to stand up 3 consecutive times from seated position. TUG was evaluated as the time it took the patient to stand up from standard chair with arm rest, walk 3 meters, and return to the chair with without assistance. Stair climbing was evaluated as the time it took the patients to go up and down 4 steps. ALSFRS-R and FVC were evaluated following standard procedures. Change in functional outcome measure was calculated as percent change from baseline.

Results: STS and SC decrease significantly faster than the 25FWT, TUG ALSFRS-R over the 9 month period. This observation is separately validated and supported by faster decline in the stair than the walking ALSFRS-R sub scores. ALSFRS-R respiratory sub score was slightly faster and declined more uniformly than measured FVC.

Discussion and conclusions: Staging of ALS is one of the determinant factors in selecting appropriate outcome measures to evaluate progression and interventions. Different components of the motor function are lost at different rates depending on the stage of ALS. In ambulatory ALS patients, the loss of the ability to stand up and climb stairs ('antigravity functional activities') are lost faster and earlier than walking. Antigravity activities like climbing stairs and standing up require a greater number and larger motor units to work which are known to be lost earlier; this may be the reason for faster progression in ALS compared to just walking. These functional outcome measures may be used to evaluate intervention in the early stage of the disease, and for small pilot studies in phase I or II clinical trials. In a large, long period efficacy trials (ie Phase III trials), the ALSFRS-R scale seems to be superior and include items that appropriately reflect changes in most aspects of motor function.

P179 ADMINISTERING AND INTERPRETING A CLINICAL BULBAR SCALE FOR ALS

BALL L[1], PATTEE G[2]

[1]*East Carolina University, Greenville, NC, United States,* [2]*University of Nebraska Medical Center, Omaha, NE, United States*

E-mail address for correspondence: balll@ecu.edu

Keywords: bulbar, clinical evaluation, dysarthria

Background: The CBS is designed to quickly and effectively assess bulbar functioning of PALS in early stages and throughout disease duration. In addition to the clinical detail of this bulbar scale, its intended design is for use across multiple clinic visits to objectively monitor the progression of bulbar motor (speech and swallowing) features in ALS clinical trials. Critical to identification of bulbar symptoms is specification of features characteristic of upper motor neuron (UMN) and/or lower motor neuron (LMN) impairment. In many cases, the ALS Team speech-language pathologist (SLP) evaluates speech and swallowing function during each clinic visit, however, these data often are reported in summary or descriptive form, with few objective results/data documented. The CBS considers interactions between ALS and contextual factors, defining levels of disability including body structures (lips, tongue, palate, pharynx), functions (speech, swallowing), activities (intelligibility, rate, intonation, naturalness, aspiration risk, inefficient dietary intake), and participation (communication effectiveness, community interactions, social networks/dining, employment/volunteer activities, telephone use).

Methods: Individual protocol items will be presented with data illustrating change with specific data points (eg, speaking rate, intelligibility, communication effectiveness). The relationship between speaking rate and intelligibility across time illustrates a non-normal trajectory but consistent change among bulbar and spinal onset ALS. Participants with ALS (n = 133) completed CBS components and results will be presented in this session.

Results: These data indicate a potential need for augmentative communication interventions when speaking rate reaches the 120–130 words per minute level, regardless of current level of intelligibility. Kaplan Meyer survival functions calculated to illustrate survival of functional speech indicate an abbreviated survival of only 308 days for individuals with spinal onset and 123 days with bulbar onset. Communication effectiveness ratings by persons with ALS and communication partners illustrate agreement in perceived limitation and that PALS experience reductions in communication effectiveness even while intelligibility remains relatively unimpaired (90–95%).

Discussion and conclusions: Indications for use of the CBS include consistent quantification of clinical bulbar data, which may be obtained in approximately 20 minutes. The data may be used clinically to predict need for interventions such as augmentative/alternative communication and PEG. The data may also be collected longitudinally to assess change in bulbar functions, rate of change, and slope of change within individual subjects or across a larger population of persons with ALS.

P180 BULBAR-RESPIRATORY ALS

CONTE A[1], DEL GRANDE A[1], LUIGETTI M[1], MADIA F[4], MELEO E[2], TONALI PA[1,3], SABATELLI M[1]

[1]*Catholic University, Department of Neurosciences, Institute of Neurology, Rome, Italy,* [2]*Catholic University, Department of Respiratory Physiology, Rome, Italy,* [3]*Don Carlo Gnocchi Foundation, Rome, Italy,* [4]*Complesso Integrato Columbus, Rome, Italy*

E-mail address for correspondence: msabatelli@rm.unicatt.it

Keywords: prognosis, bulbar onset, phenotype

Background: In about 25% of ALS patients the disease starts with bulbar symptoms and signs. Though Progressive Bulbar Palsy is no longer regarded as a distinct entity, bulbar-onset ALS shows peculiar clinical features, including older age of onset, female predominance and worse outcome. Any bulbar feature at onset and older age are consistently reported to be independently associated with poorer survival. There are several explanations for shorter survival in bulbar-onset ALS, including early respiratory muscle involvement, aspiration pneumonia, malnutrition, older age.
We have observed in some patients a 'Bulbar-respiratory' phenotype, characterized by bulbar onset ALS with early and severe respiratory involvement.

Objective: The aim of our study is to analyze clinical features of ALS patients with 'Bulbar-respiratory' phenotype.

Methods: We reviewed our ALS population with a bulbar onset and we selected those patients who had severe respiratory involvement (FVC<50% of the predicted value) without or with very slight (MRC>4) limb weakness during the follow-up period.

Results: In our series of 705 sporadic ALS patients, 206 (29.2%) had a bulbar onset. Twenty nine patients (14%) developed the Bulbar-respiratory phenotype. In patients with this subtype, the age of onset ranged from 45 to 83 years (mean 67.8, median 69); the male-to-female ratio was 1.4; the median survival was 25 months. In the remaining 177 bulbar onset ALS the mean age of onset was 62.3 years (P=0.01) and the median was 65 (range 24-84); the male-to-female ratio was 0.72; the median survival was 33 months. Using Kaplan-Meyer survival analysis we observed that disease duration was significantly shorter in 206 bulbar onset ALS with respect to 498 spinal onset ALS patients (32 months vs 43 months, P=0.009). However when bulbar-respiratory patients were excluded from the bulbar onset ALS, this difference was not statistically significant.

Discussion and conclusions: Patients with bulbar-respiratory phenotype disclosed different age of onset and gender distribution with respect to classic bulbar onset ALS, suggesting a distinct entity. Our data suggests that early respiratory involvement in a subgroup of patients is the main contributor

to worse prognosis in bulbar onset ALS. Motor neuron degeneration in ALS is an orderly and sequential process, most likely spreading from one region to contiguous anatomic ones. In the 'Bulbar-respiratory' ALS phenotype, neuronal degeneration starts in bulbar motor neurons and spreads in a rostrocaudal direction to involve phrenic nuclei and the intercostal muscles sparing those of the upper limbs. This peculiar pattern of spread suggests a primary involvement of the limbic motor control system which projects to the motor neurons innervating bulbar and respiratory muscles, throughout the Periaqueductal Gray (PAG) and the nucleus retroambiguous (NRA).

P181 LIMB DOMINANCE AND LATERALITY OF ONSET IN ALS: A PATHOGENIC ROLE FOR EXERCISE OR CLUE TO A CORTICAL VULNERABILITY?

TURNER M[1], WICKS P[2], BROWNSTEIN C[2], MASSAGLI M[2], TORONJO M[3], TALBOT K[1], AL-CHALABI A[3]

[1]*Oxford University, Oxford, United Kingdom,* [2]*Patientslikeme.com, Boston, United States,* [3]*King's College London, London, United Kingdom*

E-mail address for correspondence: martin.turner@clneuro.ox.ac.uk

Keywords: onset, spread, handedness

Background: Attempts to identify whether physical exercise is implicated in the aetiology of ALS have provided inconsistent results. Hyper-stimulation of SOD1 rats accelerated motor neuron death and induced contiguous spread of disease (1). If physical use of a limb were important in defining the site of onset then handedness might be expected to influence the side of upper limb-onset disease, and footedness likewise in lower limb-onset ALS.

Objectives: To test the null hypothesis that concordance of handedness and footedness is unrelated to laterality of upper limb or lower limb onset respectively.

Methods: ALS patients registered with an internet-based support site were invited to complete an online questionnaire concerning site of onset of symptoms, and their dominant hand and foot. A binomial test of proportions was used.

Results: A total of 343 ALS patients with limb-onset disease were studied. For upper limb-onset patients the concordance for side of onset and handedness was high (64%; $P<0.0006$). For lower limb-onset patients concordance for side of onset and footedness was absent.

Discussion: This is potentially consistent with the hypothesis that exercise influences pathogenesis in ALS, since routine physical demands on the upper limb are heavily influenced by limb dominance, whereas in the lower limbs the commonest function is standing or locomotion, using both legs equally.

There may also be a cortical vulnerability underlying upper limb-onset laterality. The 'split hand' phenomenon is postulated to reflect cortical organisation (2), presumed to be part of the evolution of fine hand control (3). There is evidence for greater connectivity in the dominant motor cortex with respect to handedness. In relation to the observations of early cortical hyperexcitability in pre-symptomatic ALS patients (4), a study in relation to handedness in healthy subjects demonstrated a shorter cortical silent period (reflecting reduced inhibitory function) in the dominant hand (5).

Conclusions: Exercise may influence site of onset in ALS, but the neocortical connectivity involved in the evolution of human dexterity may also harbor a specific vulnerability to the ageing motor system.

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P182 LOWER EXTREMITY AMYOTROPHIC DIPLEGIA: PREVALENCE AND PATTERN OF WEAKNESS

DIMACHKIE M[1,2], MUZYKA I[1], BAROHN R[1,3], KATZ J[4], JACKSON C[5], KISSEL J[6], ZHAO X-S[6], WANGY[1], MCVEY A[1], DICK A[1], PASNOOR M[1], MOZAFFAR T[7], ENSRUD E[8], ROSENFELD J[9]

[1]*The University of Kansas Medical Center, Kansas City KS, United States,* [2]*The University of Texas-Houston, Houston TX, United States,* [3]*The University of Texas-Southwestern, Dallas TX, United States,* [4]*California Pacific Medical Center, San Francisco CA, United States,* [5]*The University of Texas-San Antonio, San Antonio TX, United States,* [6]*The Ohio State University Medical Center, Columbus OH, United States,* [7]*The University of California-Irvine, Orange CA, United States,* [8]*The Boston VA Medical Center, Boston MA, United States,* [9]*Carolinas Medical Center, Charlotte NC, United States*

E-mail address for correspondence: mdimachkie@kumc.edu

Keywords: lower extremity amyotrophic diplegia, progressive muscular atrophy

Objective: To identify the prevalence of lower extremity amyotrophic diplegia (LAD) at a US academic center, describe the pattern of weakness, and provide comparative data from 8 additional major US academic institutions.

Background: We previously described LAD, a leg onset variant of progressive muscular atrophy (PMA). LAD weakness is confined to the legs for at least 2 years and there are no upper motor neuron signs.

Design and methods: Observations from nine US academic institutions were combined resulting in the identification of 26 patients with the LAD presentation. We analyzed patient medical records at the University of Kansas Medical Center (KUMC) with the lower motor neuron (LMN) presentation, focusing on 10 LAD cases.

Results: Of 318 subjects identified in the KUMC Neuromuscular Research Database, 82% (260) had amyotrophic lateral sclerosis (ALS), 1.9%(6) had familial ALS, 6.6% (21) had primary lateral sclerosis, and 31 had LMN disease. Of these 31 cases, 16 had PMA, 5 had brachial amyotrophic diplegia, while 10 had LAD. The mean LAD age of onset was 59 years with a male/female ratio of 2.3/1. Onset was asymmetric in 8/10, without side predilection. We identified a pelviperoneal pattern of weakness (sparing of knee extension and/or ankle plantar flexion) in 5 cases, diffuse leg weakness in 2, and distal predominant weakness in 3. All 10 patients had electrodiagnostic findings consistent with motor neuron disease confined primarily to the lower extremities. We also present data comparing the KUMC LAD cases to those from other

major academic neuromuscular centers. At last follow up, the weakness progressed to involve the arms in 7/26 LAD cases. During the follow up, 2/26 cases died from progression to typical ALS. Overall, the mean survival of LAD cases is 96 months.

Discussion: Marie-Patrikios described the pseudopolyneuritic form of ALS in 1918 which is a LMN syndrome confined to legs. We provided a modern description of this syndrome in 2002 and coined the term LAD. Wijesekera *et al* in 2009 described the flail leg syndrome (FLS) which is characterized by progressive distal leg weakness and atrophy. Their case definition is somewhat different from LAD since FLS cases may have pathologic stretch reflexes and remain confined to the legs for 1 year. LAD represents 3.5% of motor neuron disorders at KUMC which is comparable to the 3–6.3% described by Wijesekera *et al*. Both LAD and FLS predominantly affect men. The mean survival from the London series, Melbourne series and our series are respectively 76, 91 and 96 months.

Conclusions and relevance: The natural history of LAD differs from typical forms of ALS and PMA. LAD is a slowly progressive disorder that accounts for a third of LMN disease cases. An asymmetric pelviperoneal pattern of weakness should heighten the suspicion of LAD.

P183 BODY MASS INDEX AND SURVIVAL IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

KOSTIC S[1], STEVIC Z[2], DEDIC V[3], LAVRNIC D[2], STOJANOVIC RAKOCEVIC V[2], BASTA I[2]

[1]*CHC Zvezdara, Belgrade, Serbia,* [2]*CHC Serbia, University, Belgrade, Serbia,* [3]*Technical Faculty, University, Kragujevac, Serbia*

E-mail address for correspondence: svetlana.kostic.dedic@gmail.com

Keywords: BMI, hyperlipidemia, survival

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder. Median survival from symptom onset is about 3.5 years, but some patients survive more than 5 years. Hyperlipidemia has been reported to have a protective effect in ALS patients but not in all studies.

Aim: To determine the status of lipids in patients with ALS and investigate whether lipid contents may have an impact on disease progression and survival.

Method: Blood concentrations of triglycerides, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) and body mass index (BMI) were measured in 323 ALS patients. All patients were diagnosed as probable or definite ALS according to revised El Escorial revised criteria, at the Institute of Neurology, Belgrade, Clinical Centre of Serbia. Survival was assessed by Kaplan-Meier method.

Results: Among 323 ALS patients (193 males and 130 females, with average age 58.0±10.3), 240 (74.3%) had spinal and 83 (25.7%) patients had a bulbar onset. The mean survival time from symptom onset for ALS patients with normal lipidemia, was 2.76±1.53 years. The mean survival time from symptom onset for ALS patients with hyperlipidemia, was 3.37±2.14 years. Between these two groups we did not find a statistically significant difference (P=0.22). ALS patients with abnormally elevated LDL/HDL ratio did not have significantly longer survival in comparison to the group with normal LDL/HDL ratio (3.23±1.67 vs. 2.76±1.53, P= 0.42). Higher BMI index has not statistically significant protective role in survival (4.32±3.44 vs. 3.60±2.07, P= 0.91)

Conclusion: We did not find that ALS patients with hyperlipidemia presented longer survival. Respiratory impairment is related to a decrease in blood lipids but further studies are needed to explore this finding.

P184 ALS PATIENTS ON MECHANICAL VENTILATION HAVE GREATER RISK OF CHOLECYSTITIS

OGINO M[1], KITAMURA E[1], OGINO Y[2], MOCHIZUKI H[1]

[1]*Kitasato University School of Medicine, Department of Neurology, Sagamihara, Kanagawa, Japan,* [2]*Toshiba Rinkan Hospital, Sagamihara, Kanagawa, Japan*

E-mail address for correspondence: mieko@kitasato-u.ac.jp

Keywords: cholecystitis, mechanical ventilation

Background: We encountered several ALS patients using TPPV who experienced cholecystitis. Two of them experienced recurrence. Thus we speculated that ALS patients on TPPV might have greater risk of cholecystitis.

Objectives: To elucidate whether ALS patients on TPPV have a greater risk of cholecystitis. If so, to identify the factors that are responsible for this risk increase.

Methods: Seventeen ALS patients on TPPV and as a control group 9 patients with other neurological diseases on TPPV (4 muscular dystrophy, 1 Pompe disease, 2 central nuclear myopathy, 1 chronic progressive external ophthalmoplegia and 1 Myasthenia Gravis) and 28 long term bed ridden patients with Parkinsonism not on TPPV were analyzed. The diagnosis of cholecystitis was made based on 'Diagnostic criteria and severity assessment of acute cholecystitis: Tokyo Guidelines'. Physical examination, blood test (WBC, CRP, GGTP, ALP) and imaging examinations (at least one of following: abdominal echogram, abdominal CT, abdominal MRI, bile duct system scintigraphy) were performed.

Results: 1) Seven out of 16 ALS patients on TPPV (6 male, one female) had cholecystitis. Two of them were cholecystitis sine concrement. Two patients had a gall bladder stone without cholecystitis. In contrast only one out of nine patients with other neurological diseases on TPPV had cholecystitis. 2) Two out of 28 longtime bed ridden patients with Parkinsonism not on TPPV had cholecystitis. 3) There were not clear differences in age, gender, BMI, serum cholesterol level, Diabetes Mellitus, calorie intake and duration of TPPV in ALS patients with or without cholecystitis.

Discussion and conclusions: Our results suggest that ALS patients on TPPV have greater risk of cholecystitis than patients with other neurological diseases on TPPV and longtime bed ridden patients with Parkinsonism not on TPPV. The reason for this risk increase is not clear. 1) ALS patients using TPPV who had cholecystitis did not have characteristics generally regarded as risk factors (female gender, obesity). 2) There were no differences between ALS patients using TPPV who had cholecystitis and those who did not. 3) Positive pressure ventilation has been reported as a risk factor of cholecystitis. Patients with other neurological diseases on TPPV seem to have a lower risk but the number of patients is too small and their age is much younger so it is difficult to eliminate the risk of positive pressure ventilation. 4) Autonomic dysfunction could cause cholecystitis but Parkinsonism patients who have autonomic dysfunction do not have high risk. 5) Tube feeding was reported to cause higher risk of cholecystitis but all of the patients were on tube feeding so again this could not explain the risk increase.

We conclude that although the mechanism is not clear, ALS patients on TPPV have a greater risk of cholecystitis. Many patients using TPPV have difficulty in communication, so we have to be careful to find cholecystitis before it becomes severe.

P185 INCIDENCE OF DEEP VEIN THROMBOSIS IN IMMOBILISED ALS PATIENTS

BORISOW N, LINKE P, HOLM T, MAIER A, DULLINGER J, BAHRKE R, MEYER T

Charité, Department of Neurology, Berlin, Germany

E-mail address for correspondence: andre.maier@charite.de

Keywords: deep vein thrombosis, immobilisation, duplex sonography

Introduction: Amyotrophic Lateral Sclerosis (ALS) leads to progressive, high-grade pareses of the extremities and, as a rule, results in immobilisation. Immobilisation in the context of traumas or surgical interventions coincides with a higher incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE). In up to 20% of patients with ALS, sudden unexpected death (SUD) occurs. One possible cause of SUD is DVT in combination with PE. In ALS, the incidence of DVT as a risk factor for PE has as yet been widely unknown.

Objective: To assess deep vein thrombosis in immobilised ALS patients by using duplex sonographic examination.

Method: We conducted a prospective controlled study on duplex sonographic examinations of DVT in the lower extremities. Inclusive criteria were high-grade pareses of the legs (ALS Functional Scale - ALS-FRSr scoring <2 on the subscore for gait disturbances).

Results: The duplex sonography revealed a complete and organised DVT in 3 out of 76 patients (3.9%).

Discussion: The present study represents the first prospective, controlled study on the incidence of DVT in ALS. The DVT incidence of 3.9 % ascertained herein is lower than that detected by other authors in a retrospective study in which the incidence of DVT was 5.7%. The mere presence of DVT cannot sufficiently substantiate the high incidence of SUD associated with ALS. Other underlying causes, including autonomic failure, must be considered as central events first and foremost. Our study demonstrates that the incidence of DVT in patients with ALS is higher compared with the normal population but lower compared with patients displaying post-traumatic or post-interventional symptoms. The low incidence of DVT in ALS could be due to the protracted development of lower extremities pareses and an adaptation of the coagulation system and the venous system. Therefore, it seems that a prophylactic administration of anticoagulation drugs to immobilised ALS patients is not required.

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P186 ALS ONSET AFTER A PROLONGED TREATMENT WITH VEGF RECEPTOR INHIBITOR

MOGLIA C, CALVO A, CANOSA A, GALLO S, GIACONE S, BALMA M, CAMMAROSANO S, CHIÒ A

ALS Center, Neuroscience Department, University of Turin, Turin, Italy

E-mail address for correspondence: cristina.moglia@gmail.com

Keywords: VEGF inhibitor

Background: Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen that promotes angiogenesis in response to hypoxia, in embryologic development and in pathologic conditions. Through the binding of its receptors (VEGFR1-2-3), it has neurotrophic and neuroprotective effects on neuronal and glial cells in culture and *in vivo*, and it can stimulate the proliferation and survival of neural stem cells. Through its angiogenic effects it also has been implicated in oncogenesis, and VEGF antagonists are being used in cancer treatment. One of the most common VEGF antagonists is Sunitinib, an oral multi-targeted tyrosine kinase inhibitor with activity against the VEGF. Sunitinib is approved for treatment of advanced renal cell carcinoma (RCC), which is one of the most highly vascularized tumors. Consistent with its multitargeted profile, sunitinib may inhibit tumor growth, cause tumor regression, inhibit pathologic angiogenesis, and inhibit metastatic progression of cancer.

Case report: We report a case of amyotrophic lateral sclerosis (ALS) occurring in a patient with renal cancer treated with sunitinib. An Italian man had an history of right-sided RCC, diagnosed in 2000 and surgically excised by a right radical nephrectomy. During 5 years of oncologic follow-up there was no evidence of recurrence. In December 2005 a total body computed tomography (CT) showed lung and cerebral lesions; therefore he was started on an oral antitumoral therapy with sunitinib (50 mg qod). In October 2006, at the age of 62, he developed rapidly progressive wasting and weakness in upper limbs, associated with dysarthria and followed by dysphagia. Three months later he was evaluated in our ALS Center. The clinical examination and the electromyographic findings suggested that he had a definite ALS, according to El Escorial criteria. He still had been taking the Sunitinib. In subsequent months, he developed lower limb dysfunction, bulbar palsy and respiratory muscle insufficiency. He died on February 2008 from acute respiratory failure, 16 months from the onset of limb weakness.

Discussion: There is reasonably convincing evidence suggesting a role for VEGF and its receptors (VEGFR1, VEGFR2 and VEGFR3) in ALS. In particular, VEGF has been shown to protect motor neurons against several insults thought to be important in the pathogenesis of ALS. Reduction in activity of VEGF, through the inhibition of VEGFR1, VEGFR2 and VEGFR3, using an antitumoral as Sunitinib, might play an important role in the development and the fast course of ALS.

P187 A POSTERIORI CONFORMAL RADIOTHERAPY USING 3D DOSIMETRIC RECONSTITUTION IN ADULT-ONSET HODGKIN SURVIVOR FOR DEFINITIVE DIAGNOSIS OF A LOWER MOTOR NEURON DISEASE

PRADAT P-F[1], DELANIAN S[2]

[1]*Department of Neurology, Pitié-Salpétrière Hospital, Paris, France,* [2]*Department of Radiotherapy and Oncology, Saint-Louis Hospital, Paris, France*

E-mail address for correspondence: pierre-francois.pradat@psl.aphp.fr

Keywords: radiotherapy, Hodgkin, dosimetry

Background: Radiation-induced Lower Motor Neuron Disease (LMND) is a rare entity; its diagnosis is often difficult and may be possible only after follow-up excluding primary degenerative LMND.

Objective: To demonstrate the utility of a new method for the diagnostic of radiation-induced LMND: a *posteriori* conformal radiotherapy using 3D dosimetric reconstitution.

Method: Case report.

Results: A 47-year-old woman was referred in 2005 for a two-year progressive weakness of the lower limbs. The patient had been treated in 1993 for advanced-stage IV Hodgkin's disease with chemotherapy then radiation therapy. A supra-mediastinal mantle field followed by a subdiaphragmatic inverted Y-field including periaortic-splenic and ilio-inguinal nodes delivered 45 Gy by a 10-MV x-rays linear accelerator using equal weighting of less than 2 Gy daily fraction from anterior and posterior portals. From September 2003, she progressively developed a right, then bilateral lower limb (LL) weakness, with lower motor neuron signs and was initially diagnosed with primary muscle atrophy. Electromyography showed denervation features consistent with purely motor bilateral involvement of the L4 to S1 roots. Spinal MRI with gadolinium injection was normal. Verification of the 2D dose-surface distribution at axis made in 1999 showed no technical error, especially with a planned underdosing fields junction.
To determine if RT may have potentially injured the lumbar cord and nerves in the whole irradiated volume, *an 'a posteriori'* conformal radiotherapy with a technical reconstitution on CT-scan was performed in 2009: it showed that (a) the whole spinal cord including lumbar cord, cauda equine and plexus nerves were involved in the radiotherapy treated volumes, (b) the 3D dose-volume distribution was heterogeneous with a maximum delivered dose higher than the 45 Gy-prescribed: an unexpected 51 Gy-isodose appeared on 7 cm long and a mean 50 Gy involved 40% of the whole spinal cord.

Conclusion and discussion: Our '*a posteriori*' 3D radiotherapy dosimetric reconstruction supported post-radiation LMND diagnosis, demonstrating the anatomical structures concerned by a neurotoxic heterogeneous radiation dose-volume. The importance of an early diagnosis is justified by the emergence of new treatments. We recently reported the efficacy of PENTOCLO combination in two patients with radiation-induced LL neuropathy with predominantly motor signs. These results led us to end the phase II clinical trial of PENTOCLO combination and plan a randomized trial.

P188 A CASE OF AMYOTROPHIC LATERAL SCLEROSIS COINCIDENT WITH FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

DULLINGER JS, MAIER A, LINKE P, BORISOW N, HOLM T, MEYER T

Charité – Universitätsmedizin, Berlin, Germany

E-mail address for correspondence: joern.dullinger@charite.de

Background: Amyotrophic lateral sclerosis (ALS) and facioscapulohumeral muscular dystrophy (FSHD) are two rare neuromuscular diseases. We present a patient in whom we could detect typical features of both ALS and FSHD. Careful examinations point to a rare comorbidity of both diseases.

Case Report: In his mid-twenties, our patient developed a slowly progressive paralysis with a scapuloperoneal distribution. At the age of 59, there was a sudden progression of the paresis affecting the distal forearm extensors. At that time, a molecular genetic analysis showed a pathognomonic shortening of the repeat at the D4Z4 locus. Bearing in mind that there is a positive family history, FSHD was diagnosed.
Follow up examinations revealed a rapid decline in muscle strength resulting in a severe tetraparesis. In the neurological examination, a combined degeneration of the upper and lower motor neuron was now evident. The El Escorial Criteria for definite ALS were fullfilled and ALS was diagnosed. The patient died at the age of 61. Subsequent autopsy confirmed the clinical diagnosis of ALS showing characteristic TDP43 inclusions. Morphological changes in muscle biopsy typical of FSHD could not be detected.

Discussion: FSHD and ALS are rare neurological diseases for which different disease mechanisms are discussed. Up to now, there has been no known pathophysiological overlap between both diseases. In our patient we were able to gather several findings that suggest a comorbidity of ALS and FSHD despite its low likelihood. We also considered a pure early-onset ALS that might be associated with a D4Z4 mutation. However, taking into account all our findings, this possibility seems rather unlikely to us. Nevertheless, we cannot definitely exclude a D4Z4 mutation as a risk factor for ALS, as ALS is a multifactorial disease.
Funded by the Air Berlin Fund for ALS therapy research at the Charité.

P189 MAGNETIC RESONANCE IMAGING OF HIRAYAMA DISEASE – A MIMIC CONDITION OF MOTOR NEURON DISEASE

BEDE P[1,2], HARDIMAN O[1,2]

[1]*Trinity College, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland*

E-mail address for correspondence: bedepeter@hotmail.com

Keywords: Hirayama disease, MRI, monomelic amyotrophy

Background: Hirayama disease is a monomelic amyotrophy, a benign condition of the cervical spinal cord that can mimic motor neuron disease (MND). It frequently presents with unilateral or asymmetrical upper limb weakness and atrophy in young male patients. Electrophysiological studies are not specific to the condition. Anterior displacement of the posterior wall of the cervical dural canal is thought to be the underlying pathomechanism. The recognition and establishment of the diagnosis of Hirayama disease is challenging because of

confounding clinical and electrophysiological features similar to MND. Early diagnosis and prevention of flexion by a collar might prevent disease progression.

Method: Four patients with Motor Neuron Disease, 4 age-matched healthy controls and 2 young patients with suspected Hirayama disease underwent cervical spinal cord imaging. A 3.0 Tesla MRI system was used to acquire 3D high resolution structural and diffusion tensor data of the cervical spinal cord in extension and flexion. Multi-echo fast field echo (mFFE) MRI sequence was used to enhance spinal grey matter - white matter contrast.

Results: The clinical suspicion of Hirayama disease was confirmed by demonstrating marked anterior shifting of the posterior wall of the cervical dural canal with cord flattening in flexion. No similar changes have been observed on the healthy controls and motor neuron disease patients.

Conclusion: Standard non-flexion, neutrally positioned cervical spinal cord MRI might be non-specific to Hirayama disease. Dynamic myelography or flexion MRI studies are required to confirm the diagnosis.

P190 TRANSCRANIAL PARENCHYMAL SONOGRAPHY IN AMYOTROPHIC LATERAL SCLEROSIS

STEVIC Z[1], PAVLOVIC A[1], KUZMANOVIC A[1], DEDIC V[2], LAVRNIC D[1]

[1]*Institute of Neurology, Belgrade, Serbia,* [2]*Techical Faculty, University of Kragujevac, Kragujevac, Serbia*

E-mail address for correspondence: zsmnd.yu@sezampro.rs

Keywords: transcranial, ultrasonography, substantia nigra

Background: There is accumulating evidence that amyotrophic lateral sclerosis (ALS) is a multisystem degenerative disease, raising the question whether some clinical parkinsonism features are of extrapyramidal origin. Enlargement of the echogenic signal (hyperechogenity) of the substantia nigra (SN) registered by transcranial sonography (TCS) of the brain structures, has been reported a highly characteristic finding in idiopathic Parkinson's disease. TCS has not been used to assess brainstem and subcortical brain structures in ALS until now.

Aims: To investigate possible degeneration of basal ganglia in sporadic ALS (SALS) patients using TCS, and its clinical correlates.

Methods: Thirty one nondemented patients, with probable or definite ALS, according to El Escorial criteria, (19 women and 12 men, average age 59.90±8.17) were compared to 31 age-matched controls (19 women and 12 men, average age 55.22±10.89). Twenty three (74.1%) had spinal onset and 8 (25.9%) had bulbar onset of the disease. The mean duration of the disease was 2.55±2.46 (range 0.5-10 years). For TCS examinations, a color-coded phased array ultrasound system equipped with 2.5 MHz transducer (ALOK, Alpha 10, Japan),was performed through a preauricular acoustic window, with a penetration depth of 16 m and dynamic range of 45–50 dB. The ALSFRS-r was an instrument for evaluation of the functional status of patients with ALS.

Results: Unilateral SN hyperechogenity was identified in 6 (19.35%) examined ALS patients, which was marked in 2 (6.45%) and moderate in 4 (12.90%) patients. Unilateral SN marked hyperechogenity was found in 1 (3.22%) control. The mean SN echogenity was not significantly different between groups. Between the mean SN echogenity and ALSFRS-r score we registered a statistically significant negative correlation ($P = 0.009$).

Conclusion: Our pilot study did not show significant impairment in SN in SALS patients examined with TCS.

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THEME 11 IMAGING, ELECTROPHYSIOLOGY AND MARKERS OF DISEASE PROGRESSION

P191 TRANSCRANIAL SONOGRAPHY OF THE SUBSTANTIA NIGRA IN ALS: PILOT DATA

FATHINIA P, KASSUBEK J, LUDOLPH AC

University Hospital Ulm, Ulm, Germany

E-mail address for correspondence: panteha_fathinia@gmx.at

Keywords: transcranial sonography, substantia nigra, symptomatic therapy

Background: Transcranial sonography is a fast and fully non-invasive technique for the depiction of the basal ganglia, as already extensively assessed in Parkinson´s disease.

Objectives: The idea of this pilot study was to examine if there is a link between morphological alterations, ie hyperechogenicity of the substantia nigra and ALS as a correlate of associations between the clinical finding of coexistent rigidity in motor neuron disorders.

Methods: Thirty-three patients (aged 38 to 78, 17 m/16 f) with the diagnosis of possible, probable or definite ALS were examined with trancranial sonography. The hyperechogenic area of the mesencephal brainstem was measured planimetrically. Thus, hyperechogenicity of the substantia nigra was defined by an increased size of this region compared both with literature (1) values and compared with a healthy control group (18 patients, aged 27 to 75, 10 m/8 f). We defined hyperechogenicity as a planimetric measurement result of more than 0.20 cm^2, as also used in Parkinson´s disease.

Results: Twenty-three ALS patients showed a significant bilateral hyperechogenicity of the substantia nigra with planimetric measurements over 0.25 cm^2. Five patients showed no changes compared to controls and five other patients could not be assessed due to insufficient bone window. The controls had a median hyperechogenic area size of 0.1 cm^2.

Discussion: Out of 33 patients with the diagnosis of ALS examined via transcranial sonography, 23 patients showed a hyperechogenicity of the substantia nigra. These pilot results will await confirmation by examination in a larger patient collective.

Conclusions: By sonographic substantia nigra assessment, it might be possible to establish a correlation between morphological alterations of the substantia nigra and the symptomatic therapeutic value of L-Dopa in ALS.

Reference:

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P192 QUANTITATIVE SENSORY TESTING DEMONSTRATES C-FIBRE, A-Δ FIBRE, AND A-β-FIBRE INVOLVEMENT IN ALS

PONFICK M, KLUG R, GDYNIA H-J, GASTL R, KASSUBEK J, SPERFELD A-D, LUDOLPH AC

University of Ulm, Department of Neurology, Ulm, Baden-Württemberg, Germany

E-mail address for correspondence: matthias.ponfick@uni-ulm.de

Keywords: quantitative sensory testing, small-fibre neuropathy, somatosensory nerve system

Background: Although the hallmark of ALS is a progressive degeneration of the central and peripheral motor system, variable deficits of the somatosensory nervous system have repeatedly been described.

Objective: The aim of this study was to examine the potential of quantitative sensory testing (QST) in order to further substantiate a sensory involvement in ALS.

Methods: Twenty-one patients with ALS (mean age 62.4±10.7 years, mean disease duration 41.9±34.6 months, mean ALSFRS-R 30.95±10.9) were tested with QST using a standardized and validated protocol.

Results: Clinically, only 9/21 (0.43, 95 CI: 0.23- 0.66) patients showed sensory disturbances, whereas the QST revealed distinct abnormalities in 20/21 (0.95, 95 CI: 0.76-1.0) patients. Nineteen patients (0.90, 95 CI: 0.70-0 .99; 2x 19/21) showed abnormalities in at least one parameter which are representing C-fibre and A-δ fibre function. Twelve patients (0.57, 95 CI: 0.34-0.78) presented at least one pathologic value in A-β-fibre parameter and fourteen (0.67, 95 CI: 0.43-0.85) showed central pain processing disorders.

Discussion: By using QST in ALS patients, we detected (sometimes subtle) somatosensory nervous system involvement in the majority of patients. Currently, further investigations concerning histological examinations of skin nerve biopsies, in order to analyse potential small-fibre involvement are ongoing.

Conclusions: The QST seems to be an appropriate method to detect sensory fibre involvement in ALS.

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P193 SLOW SACCADES IN BULBAR-ONSET MOTOR NEURONE DISEASE

DONAGHY C[1], PINNOCK R[1], FORBES R[1], HARDIMAN O[2], PATTERSON V[1], MCGIVERN CR[1], GIBSON MJ[1]

[1]*Royal Victoria Hospital, Belfast, United Kingdom,* [2]*Beaumont Hospital, Dublin, Ireland*

E-mail address for correspondence: donaghy1a@hotmail.com

Keywords: saccades, ocular fixation, PSP

Background: Current findings suggest that eye movement abnormalities in ALS relate to frontal lobe impairment. Numerous case reports, however, describe slow saccades and supranuclear gaze palsies in patients with MND often associated with bulbar-onset disease. We performed a study of saccades and ocular fixation in patients with MND to examine for any differences between bulbar-onset, spinal-onset ALS and controls. We then compared the results for bulbar-onset ALS with a group of patients with PSP.

Methods: Forty-four ALS patients, 45 controls and 7 PSP patients were included. Reflexive horizontal saccades (latency and speed) and ocular fixation (saccadic intrusion amplitude and frequency) were examined using infra-red oculography. A Saccadic Intrusion (SI) Index was developed by the authors representing saccadic intrusion frequency and amplitude.

Results: Saccades were found to be slower in bulbar-onset compared to spinal-onset patients and controls ($P=0.03$, $P=0.05$). PSP patients had significantly slower saccades compared to controls ($P=0.006$). SI frequency was greater in PSP and bulbar-onset ALS patients compared to controls ($P=0.006$, 0.015). Similarly, SI Index was greater in PSP and bulbar-onset ALS patients compared to controls ($P=0.01$, 0.002). Although not statistically significant, PSP patients had increased SI freq, SI amplitude, SI Index and slower saccades compared to bulbar-onset ALS patients.

Conclusions: This is the first study to highlight the presence of slow saccades in bulbar-onset MND. It is likely that a spectrum exists, ranging from normal saccade speed to the presence of a gaze palsy. This theory is consistent with the similarities found in the eye movement profile of PSP and bulbar-onset ALS patients. A longitudinal study would be of great interest to examine the potential of eye movements as a biomarker for ALS disease progression. In addition, SI Index appears to be a sensitive measure of ocular fixation abnormality that could be employed in further research.

P194 REDUCED MOTOR NETWORK CONNECTIVITY DURING REST IN ALS

JELSONE-SWAIN L[1], GRUIS K[2], HOVATTER R[1], SEIDLER R[4,5], FLING B[5], WELSH R[1,3]

[1]*Department of Radiology,* [2]*Department of Neurology,* [3]*Department of Psychiatry,* [4]*Department of Psychology,* [5]*Division of Kinesiolog; University of Michigan, Ann Arbor, MI, United States*

E-mail address for correspondence: rcwelsh@med.umich.edu

Keywords: resting state, fcMRI, motor cortex

Background: Neurodegenerative diseases do not manifest randomly, but affect disease-specific cortical neural networks. In ALS, observations of altered structure and function in the sensorimotor network have been reported, however the intrinsic global functioning of upper motor neurons during early disease stages is poorly understood. Furthermore, it is not known how functionally connected nodes within this network change with disease progression. Identifying these changes with resting-state functional connectivity magnetic resonance imaging (fcMRI) has important implications in understanding ALS pathophysiology and possible diagnostic implications.

Objectives: Using fcMRI during rest, our goal was to examine cortical coupling: 1) between primary motor cortices (M1) and between nodes of the entire sensorimotor network; 2) examine the sensorimotor network in high-functioning patients to delineate neural changes that may precede moderate-severe symptom presentation; and 3) identify longitudinal functional changes in these networks.

Methods: Twenty patients with ALS (11 males, < age>=59 years) within 24 months of diagnosis, and 20 age/sex-matched healthy volunteers (13 males, < age>=58 years) were scanned at the University of Michigan's MRI laboratory on a GE 3T magnet. Of these 20, nine longitudinal ALS participants were scanned again an average of nine months later. fcMRI image acquisition lasted six minutes during rest. Two network analyses were performed to directly examine: 1) interhemipsheric M1 coupling and 2) whole sensorimotor network connectivity. **M1 coupling:** Anatomical masks were created along the precentral gyrus in each hemisphere and then segmented into forty regions of interest (ROI), thus increasing sensitivity to discretely localized regions that may be affected by ALS. **Sensorimotor connectivity:** Single ROIs in the dorsal and ventral premotor cortex, supplementary motor area (SMA), pre-SMA, M1, and somatosensory cortex in each hemisphere were created. Correlation strength and number of significant connections between ROIs for each analyses were compared between groups and scanning sessions.

Results: The ALS group showed significantly less interhemispheric M1 connectivity than the healthy control group ($P<0.01$). A group x ROI interaction ($P<0.00001$) indicated that decreased connectivity was more pronounced in dorsal regions of M1. Within subjects analysis of the ALS group resulted in significantly reduced interhemispheric correlations at their subsequent scanning session ($P<0.005$). Examining the sensorimotor network, the number of statistically-significant connections was less in the ALS group, with 31 connections present in the healthy control group, 28 connections in the ALS group at time-point-one and 23 connections at time-point-two.

Discussion and conclusion: Results from the current study indicate that functional neural changes are occurring early, before moderate symptom presentation, and are rapidly progressing. Furthermore, the pattern of decreased network connectivity may be more specific to dorsal M1 locations, which correspond to limb and trunk regions of the body. Further study is needed to place these findings in the context of the pathophysiology of the disease.

P195 MOTOR NETWORK DEGENERATION IN ALS: A STRUCTURAL AND FUNCTIONAL CONNECTIVITY STUDY

VERSTRAETE E, VAN DEN HEUVEL MP, MANDL RC, VELDINK JH, VAN DEN BERG LH

UMC Utrecht, Utrecht, Netherlands

E-mail address for correspondence: E.Verstraete@umcutrecht.nl

Keywords: cortical thickness, diffusion tensor imaging, resting-state functional MRI

Background: Amyotrophic lateral sclerosis (ALS) is known to primarily affect motor neurons. The pattern of neurodegen-

eration and how it affects the central motor network as a whole is however, unknown.

Objectives: To integrate structural and functional imaging measures on the motor network in patients with ALS and healthy controls.

Methods: Twelve patients with ALS and twelve matched controls were studied. Cortical thickness measurements and diffusion tensor imaging (DTI) were carried out on crucial motor tracts, including the corticospinal tracts (CST) and the corpus callosum. Furthermore, these structural measures were combined with assessment of functional connectivity of the motor network based on resting state fMRI.

Results: Cortical thinning was observed in the primary motor areas in ALS patients compared to controls and was found to be associated with disease progression. Moreover, FA values were found significantly reduced in the corpus callosum and in the rostral part of the CST. Overall functional organisation of the motor network was not significantly altered, but the level of functional connectedness in patients with ALS was found to be associated with disease progression rate.

Discussion and conclusions: We demonstrate central motor network deterioration in ALS together with clinical implications of the functional connectedness of the motor network. These data corroborate the spread of disease along the functionally and structurally linked neural structures of the motor network.

P196 UPPER MOTOR NEURON ABNORMALITIES ON CONVENTIONAL BRAIN MRI IN ALS PATIENTS: CLINICAL FEATURES AND NATURAL HISTORY

MATTE G, PIORO E

Cleveland Clinic, Cleveland, OH, United States

E-mail address for correspondence: PIOROE@ccf.org

Keywords: neuroimaging, phenotype, disease progression

Background: Routine T2 and proton density (PD) sequences on conventional brain MRI (cMRI) occasionally reveal abnormalities in upper motor neuron (UMN) regions of ALS patients as corticospinal (CST) hyperintensity and primary motor cortex (PMC) hypointensity. The clinical, pathological, and prognostic significance of these changes is unknown, and distribution of CST hyperintensities is poorly defined.

Objective: Define the phenotype and prognosis associated with CST hyperintensity and/or PMC hypointensity on T2 and PD of cMRI sequences in ALS patients.

Methods: Clinical data were retrospectively reviewed from 112 patients diagnosed with ALS at the Cleveland Clinic who underwent brain MRI. Five millimeter-thick transverse brain images were obtained at 1.5T using fast spin-echo parameters: TR=3750 ms, TE=80, 20 ms.

Results: Forty six of 112 patients (41%) had PMC hypointensity and/or CST hyperintensity on a single cMRI scan performed 26 months (mean) after symptom onset. Patients with either of these changes were younger at symptom onset (53.1 vs. 58.4 years, $P=0.02$) and tended to be males (32/46 vs. 33/66, $P=0.052$). Patients with cMRI changes typically displayed predominant UMN phenotype (40/46 vs. 37/66, $P=0.001$), and rarely had an exclusively lower motor neuron phenotype (1/46 vs. 12/66, $P=0.014$). Patients with CST hyperintensity were younger at symptom onset than patients with isolated PMC hypointensity (50.9 vs. 60.0 years, $P=0.03$) or no MRI abnormalities (50.9 vs. 58.4 years, $P=0.005$). Patients with CST hyperintensity had a shorter survival than patients with PMC hypointensity (3.5 vs. 7.0 years, $P=0.004$). When present, CST hyperintensity occurred at the cerebral peduncle (CP) level in 35/35 patients and in a contiguous fashion if visible rostrally. Patients with both PMC hypointensity and CST hyperintensity were more likely to have hyperintensity extending to the immediate subcortical white matter than patients with only CST hyperintensity (10/17 vs. 3/18, $P=0.015$). However, these two groups were similar in clinical phenotype and prognosis.

Discussion: We demonstrate in a large cohort of ALS patients with long-term clinical follow-up that presence of these cMRI changes is associated with characteristic clinical features. CST hyperintensity with or without PMC hypointensity is associated with a more aggressive UMN phenotype, while PMC hypointensity alone identifies a disease course resembling that observed in the absence of cMRI changes. CST hyperintensity is most likely seen at the CP, possibly because of the compactness of fibers here, but its presence more rostrally may represent either retrograde UMN degeneration or a different pathogenic process.

Conclusions: ALS patients with CST hyperintensity and/or PMC hypointensity develop UMN-predominant disease at a younger age than patients without such cMRI changes. Whether this indicates differences in pathogenic mechanisms or UMN degeneration requires further study. Identifying ALS subgroups with distinct clinical and prognostic features using cMRI may be useful for patient stratification in ALS clinical trials.

P197 VOXEL-BASED MORPHOMETRY AND FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH BEHAVIORAL IMPAIRMENT

TROJSI F[1,2], ESPOSITO F[2,3], SAGNELLI A[1], PACCONE A[2], CORBO D[2], PICCIRILLO G[1], TEDESCHI G[1,2], MONSURRÒ MR[1]

[1]*Department of Neurological Sciences, Second University of Naples, Naples, Campania, Italy,* [2]*Neurological Institute for Diagnosis and Care "Hermitage Capodimonte", Naples, Campania, Italy,* [3]*Department of Neuroscience, University of Naples "Federico II", Naples, Campania, Italy*

E-mail address for correspondence: francesca.trojsi@unina2.it

Keywords: resting-state functional magnetic resonance imaging (RS-fMRI), voxel-based morphometry, default-mode network (DMN)

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, mainly characterized by the progressive degeneration of upper and lower motor neurons, but does not spare extramotor areas, causing cognitive and behavioral syndromes.
To better investigate structural and functional abnormalities in ALS, we used an optimized version of voxel-based morphometry (VBM) analysis combined to functional resting-state fMRI data in a population of behaviour impaired patients.
Resting-state functional magnetic resonance imaging was performed at 3 Tesla on 20 ALS patients with frontal dysfunction and 20 age- and sex-matched healthy volunteers. Resting-state network maps, extracted with independent component analysis and group-level statistical analyses, were performed to detect disease and disease-by-age interaction effects. Using the high quality 3D-T1 whole-head scans available for all 40 subjects, we conducted an optimized voxel-based

morphometric analysis, importing 3D-T1 scans to the SPM8 software package. Then, to allow voxel-by-voxel comparisons with network maps in the same anatomical space, the series of segmented grey matter (GM) images were transformed to the Talairach space.
The sensori-motor network showed significant disease effects, with signal suppression in patients in the primary and the supplementary motor cortices, principally in the left hemisphere. The same was visible frontally in the right fronto-parietal network, possibly reflecting the patients' frontal dysfunction. The default-mode network showed no group differences, but a significant disease-by-age interaction in the posterior cingulate cortex, where signals positively correlated with age and disease duration and negatively correlated with the functional rating scale score in patients. Similar effects were detected within the right fronto-parietal network. Compared with controls, ALS patients had significant clusters of locally reduced GM density ($P < 0.05$) in the left premotor and right fronto-parietal cortex.
We observed no spots of reduced GM in ALS patients within the default-mode network, suggesting that possible compensation mechanisms linked to the observed disease-by-age interactions in the cognitive networks are active in regions with no ongoing structural degeneration.
Therefore, the disease-by-age interaction in the default-mode and right fronto-parietal networks unravels a possible mechanism of compensation between motor and extra-motor systems. Given the striking spatial contiguity of disease effects in default and sensorimotor networks connectivity and gray matter densities values, we can speculate that the suppression of the within-network RS-fMRI signals may be an earlier marker of the structural degeneration and not vice versa, concluding with the intriguing finding that the mechanism of functional networks' rearrangements anticipates in ALS the atrophic degeneration process.

P198 WHITE MATTER TRACT DAMAGE AND COGNITIVE DYSFUNCTION IN ALS

PETTIT L, BASTIN M, ABRAHAMS S

University of Edinburgh, Edinburgh, Midlothian, United Kingdom

E-mail address for correspondence: L.D.Pettit@sms.ed.ac.uk

Keywords: imaging, cognitive, dysfunction

Background: Cognitive dysfunction in non-demented amyotrophic lateral sclerosis (ALS) has been consistently demonstrated. Tests of verbal fluency (VF) are the most commonly reported impairments and have been associated with reduced white matter volume in frontotemporal areas. Recent developments in magnetic resonance imaging (MRI) techniques allow the investigation of white matter structure and integrity.

Objectives: To investigate the extent and location of white matter damage in ALS patients and its relationship to cognitive dysfunction.

Method: Conventional and Diffusion Tensor (DT) magnetic resonance imaging data were acquired from 8 ALS patients and 8 healthy age-matched controls. A measure of mean diffusivity (MD) was extrapolated from the data and compared between groups using Tract Based Spatial Statistics (TBSS). Cognitive functioning in the ALS group was assessed by neuropsychological tests of verbal fluency, the Brixton spatial awareness test, and the graded naming test (GNT).

Results: Contrast analyses revealed significantly ($P<0.05$, corrected for multiple comparisons) higher MD in patients compared to controls in extensive prefrontal white matter regions, including regions through which run: the inferior longitudinal fasciculus (connecting the temporal and occipital lobes), the arcuate fasciculus (connecting temporoparietal and frontal areas), the uncinate fasciculus (connecting the limbic system to orbitofrontal cortex), and the cingulum. In addition, ALS patients had significantly higher MD in the internal and external capsules, as well as frontal association fibres running into superior frontal gyrus (BA 10) and dorsolateral prefrontal cortex (BA 9, 44, and 46). Results from the cognitive tasks indicated that the ALS group performed worse than controls in spoken verbal fluency (Z score = -1.8 compared to control mean). Four ALS patients had Z scores greater than -1.96 suggesting that half of the patients were significantly impaired on fluency. The ALS group performed comparably to control norms in the GNT and Brixton tests, however patients' Brixton scores were variable with two patients in the abnormal/ poor range and three patients in the superior range.

Discussion: ALS patients exhibited damage to multiple white matter tracts, as indicated by high mean diffusivity. There was a preponderance of prefrontal involvement, with tracts leading to dorsolateral, superior/medial, and orbitofrontal regions showing poor integrity. Patients within this group were found to be impaired on verbal fluency, suggesting that white matter integrity may underlie this cognitive dysfunction. Impoverished white matter integrity may be a symptom of spreading neuronal pathology or Wallerian degeneration following neuronal loss and requires further investigation.

Conclusions: The current investigation provides strong evidence of damage to white matter tracts in ALS, and suggests that white matter integrity may underpin the pattern of cognitive impairment demonstrated in a proportion of patients.

P199 NEURORADIOLOGICAL CHARACTERISATION OF EXTRA MOTOR CORTEX PATHOLOGY IN AMYOTROPHIC LATERAL SCLEROSIS

BEDE P[1,2], BOKDE A[1,2], JORDAN N[2], ELAMIN M[1,2], FAGAN A[3], HARDIMAN O[1,2]

[1]*Trinity College, Dublin, Ireland,* [2]*Beaumont Hospital, Dublin, Ireland,* [3]*St James Hospital, Dublin, Ireland*

E-mail address for correspondence: bedepeter@hotmail.com

Keywords: MRI, cognition, frontotemporal dementia

Background: Amyotrophic Lateral Sclerosis is a complex multisystem disorder with non-motor manifestations: neuropsychological and behavioural deficits. ALS with Frontotemporal Dementia (ALS-FTD) is increasingly recognized as a distinct phenotype of Motor Neuron Disease with distinguishing clinical, neuroradiological and neuropsychological features. Behavioural and cognitive deficits in ALS may precede motor symptoms, are challenging to manage and have a significant impact on survival and the quality of life of carers and patients.

Aims: The aim of this study is to determine the extent of frontotemporal pathology in ALS patients with no overt cognitive impairment and to describe the Magnetic Resonance Imaging characteristics of the ALS-FTD complex.

Methods: Thirty age matched healthy controls and 30 'definite' ALS patients according to the El Escorial criteria have been recruited. Participants are assessed by a comprehensive battery of neuropsychological tests and undergo 3 Tesla high resolution magnetic resonance neuroimaging. The neuropsychological battery includes the Boston naming test, Raven`s

standard progressive matrices, Logical Memory tests, the California verbal learning test, Verbal paired associates, the Rey Complex Figure test, the Brixton spatial anticipation test, Digit span, Verbal fluency tests, Category Fluency, the Wechsler test of adult reading, The colour word task –Stroop test, the Hospital Anxiety and Depression Scale and the Frontal Systems Behaviour Scale (FrSBe). The neuroimaging protocol includes: Voxel Based Morphometry (VBM) based on high resolution structural imaging data, Magnetic Resonance Spectroscopy (MRS), Diffusion Tensor Imaging (DTI) and Resting state Functional MRI (rfMRI). A routine Fluid-attenuated inversion recovery (FLAIR) sequence is used for the identification of underlying cerebrovascular disease to exclude patients from the research study.

Results: Five ALS patients fulfilled the Neary Criteria for Frontotemporal dementia. Fifteen ALS patients scored within normal range in all of the neuropsychological domains when compared to a pool of age matched healthy controls, and were categorized as ALS patients with no cognitive deficits. Ten ALS patients demonstrated executive dysfunction on neuropsychological testing. Voxel based morphometry (VBM) analysis demonstrated considerable differences in grey matter volumes between ALS-FTD and ALS patients with no cognitive deficits. Comparative statistical maps highlighted significant differences ($P<0.05$) in the left anterior temporal lobe, ventral medial frontal lobe and parahippocampal regions. ALS patients with no cognitive deficits showed grey matter volume changes in the hippocampus, left dorsolateral prefrontal cortex, cerebellum, posterior cingulate and in the in right superior temporal gyrus when compared to healthy controls.

Conclusions: The results underscore the heterogeneity of extra motor involvement within the ALS spectrum. The study demonstrates the unique radiological attributes of the ASL-FTD complex. However, ALS patients with no cognitive or behavioural deficits on formal neuropsychological testing also show signs of extensive extra motor cortex atrophy on a group level.

P200 CORPUS CALLOSUM INVOLVEMENT IS A CORE FEATURE OF ALS, AND MAY REFLECT EARLY INTER-HEMISPHERIC SPREAD OF PATHOLOGY

FILIPPINI N, DOUAUD G, MACKAY C, KNIGHT S, TALBOT K, TURNER M

Oxford University, Oxford, United Kingdom

E-mail address for correspondence: martin.turner@clneuro.ox.ac.uk

Keywords: corpus callosum, diffusion tensor imaging, biomarker

Background: Focus of onset and spread of pathology throughout upper (UMN) and lower motor neuron (LMN) compartments in ALS are poorly understood. Diffusion tensor imaging (DTI) is an established and sensitive MRI application for the non-invasive detection of white matter (WM) pathology, but biomarkers to improve diagnosis and therapeutic monitoring in ALS must accurately reflect the inherent clinical and prognostic heterogeneity.

Objectives: We sought to establish a core signature of cerebral WM change in heterogeneous cases of ALS with variable UMN involvement clinically, using unbiased whole-brain DTI, that might also inform onset and spread of pathology.

Methods: High-field 3T DTI was applied (using whole-brain Tract-based Spatial Statistics, TBSS) to a group of 24 unselected heterogeneous ALS patients, in comparison with very closely age and gender-matched healthy controls. Patients were scored for clinical UMN involvement and disability (using the revised ALS Functional Rating Scale, ALSFRS-R). Voxel-based morphometry of T1 images was also undertaken to explore any associated grey matter (GM) atrophy.

Results: A consistent, highly significant reduction in fractional anisotropy (FA) was demonstrated in the corpus callosum (CC) of the ALS group, extending rostrally to the motor cortices. Regional analysis of radial diffusivity increase (a DTI measure thought to reflect Wallerian degeneration), closely matched the FA regional changes. FA changes detected in the more caudal corticospinal tracts (CST) were less marked than those seen rostrally. All the FA changes (including the CC) were strikingly consistent with historical post-mortem neuropathological findings in ALS patients (1).
CC changes were independent of UMN involvement. There was a limited correlation with disability. A separate whole-brain analysis by clinical UMN involvement delineated just the CSTs. GM changes were widespread and consistent with areas of WM involvement. Post hoc discriminant analysis using the combination of FA, RD and GM values separated the two groups with 92% sensitivity and 88% specificity.

Discussion: CC involvement is a consistent feature of ALS across a range of phenotypes, independent of clinical UMN involvement, and may reflect early inter-hemispheric spread of pathology. Transcranial magnetic stimulation (TMS) studies through the CC have previously been noted to be abnormal in ALS.
The notable delineation of the CST in whole-brain analysis of FA in relation to clinical UMN involvement provided further validation of DTI as a sensitive tool for detecting neuronal pathology in ALS.

Conclusions: Our findings support an inherent cerebrally-driven pathological process, rather than simple anterior horn cell 'dying back' in ALS. CC changes may have particular potential as an early biomarker in those identified in the future to be 'at risk', possibly through a combination of DTI and TMS.

Reference:

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P201 FINGERPRINT CHARACTERISTICS OF RARE MOTOR NEURON DISORDERS BY APPLICATION OF DIFFUSION TENSOR IMAGING METHODS

UNRATH A[1], MUELLER H-P[1], SPERFELD A-D[2], LUDOLPH AC[1], KASSUBEK J[1]

[1]*University of Ulm, Department of Neurology, Ulm, Germany,* [2]*HELIOS Clinics Bad Saarow, Department of Neurology, Bad Saarow, Germany*

E-mail address for correspondence: alexander.unrath@uni-ulm.de

Keywords: DTI, pathoanatomy, biomarker

Background and objectives: Motor neuron disorders (MND) are clinically defined by the predominance of upper or lower motor neuron involvement and the course of the disease. Magnetic resonance imaging (MRI) basically serves as a diagnostic tool to exclude other pathologies, but gradually novel approaches based on automated and computer-assisted techniques, such as diffusion tensor imaging (DTI) methods help to identify the pathophysiological processes of these disorders within the cerebral white matter (WM) *in vivo*. This study was designed to investigate different rare MND in order to define pathoanatomical characteristics.

Methods: Five groups of rare motor neuron disorders were included in this study, ie primary lateral sclerosis (PLS, N=25), pure and complicated hereditary spastic paraparesis (pHSP, N=24 and cHSP, N=14, respectively), X-linked spinobulbar muscular atrophy (X-SBMA, N=20) and spinal muscular atrophy (SMA, N=21). Whole-brain based DTI analysis methods were applied to the patient groups in comparison with matched controls. All data analyses were performed by use of the DTI software *TIFT* (Tensor Imaging and Fiber Tracking).

Results: WM analysis revealed widespread affectations and characteristic patterns of alterations within the supratentorial motor system in all patient groups with varying predominance according to the clinical focus, noteworthy also in disorders with a clinically defined isolated involvement of the lower motor neuron. Further reductions of the fractional anisotropy as a marker of WM integrity were observed within distinct regions of the corpus callosum in the pHSP and particularly cHSP group as well as in PLS patients, additionally in non-motor regions in projection to the limbic system in X-SBMA and both HSP groups. The most widespread extra-motor WM affectations were observed in the cHSP group.

Discussion and conclusion: In summary, DTI was able to delineate a characteristic WM pathoanatomy in motor and extra-motor brain areas for different MND via whole brain-based fractional anisotropy assessment which provides a fingerprint identification of these disorders. Future developments should aim at multiparametric imaging analyses to explore biomarker information.

P202 RESEARCH ON THE CLINICAL FEATURES, MAGNETIC RESONANCE IMAGING AND ELECTROMYOGRAPHY OF 28 CASES DIAGNOSED WITH HIRAYAMA DISEASE

ZENG Y, HU X-H, HUANG R, FANG D-F, GUO X-Y, SHANG H-F

Department of Neurology, West China Hospital of Sichuan University, Chengdu, Sichun, China

E-mail address for correspondence: hfshang@yahoo.com

Keywords: MRI, electromyography, Hirayama Disease

Objective: To study the clinical, magnetic resonance imaging (MRI) and electromyography (EMG) features in Hirayama Disease (HD).

Methods: Twenty-eight patients with HD were included in the study. Clinical information, results of cervical MRI in a neutral and a fully flexed position, and results of EMG of ulnar nerve and median nerve in a neutral and a fully flexed position were analyzed.

Results: The 28 cases consisted of 27 males and 1 female and the mean onset age was 16.9±1.56 years. The mean delay between age at onset and age at diagnosis was 1.80±1.00 years (except for 1 case with delay of 18 years). The clinical symptoms and signs of these patients were not progressed with follow-up of 1-3 years. Twenty-five patients had symptoms and signs on one side only (left to right ratio: 8/17). Both sides were affected in 3 patients. Cervical spinal cord MRI of all patients showed typical HD MRI features. EMG tests revealed abnormalities of neurogenic changes in all clinically affected upper limbs in all patients. Clinically unaffected upper limbs of 11 patients (44%) who had unilateral clinically affected upper limbs were also found to have abnormalities by EMG test. The frequency of F wave for ulnar and median nerves of clinically affected limbs was significantly lower than that of unaffected upper limbs ($P<0.05$) whenever the neck was in a neutral or flexed position. The frequency of F wave of ulnar and median nerves when the neck was kept in a neutral position was significantly lower than that in a flexed position whenever testing the clinically affected or unaffected upper limbs ($P<0.05$). There was no significant difference in the frequency of F wave of the median nerve in clinically unaffected upper limbs between neutral and flexed neck positions ($P=0.144$). The amplitude of F wave of the median nerve in clinically affected limbs when the neck was kept in neutral position was significantly higher than that in a flexed position ($P=0.048$).

Conclusion: Adolescents presenting unilateral limb weakness and/or atrophy should be considered with Hirayama disease, and confirmed by cervical spinal MR imaging in a neutral and a fully flexed position. EMG test is sensitive to find abnormality in the clinically unaffected limb. The frequency and amplitude of F wave may be a useful index to evaluate the severity and progression of Hirayama Disease.

P203 CHANGES IN ELECTROPHYSIOLOGICAL MARKERS OF COGNITION IN ALS: PRELIMINARY RESULTS OF A HIGH-RESOLUTION EEG STUDY

STUKOVNIK V[1], DREO J[1], BON J[2,4], PODNAR S[1], ZIDAR J[1], REPOVŠ G[3]

[1]*Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia,* [2]*Psychiatric Hospital Begunje, Begunje, Slovenia,* [3]*Department of Psychology, Faculty of Arts, University of Ljubljana, Ljubljana, Slovenia,* [4]*Division of Neurology, University Medical Centre Ljubljana, Ljubljana, Slovenia*

E-mail address for correspondence: vita.stukovnik@kclj.si

Keywords: high-resolution EEG, event related potentials, cognition

Objective: Amyotrophic lateral sclerosis (ALS), traditionally considered exclusively as a motor system disorder, is increasingly recognized as a multi-system disease, affecting also non-motor areas, with results ranging form subtle behavioral and executive dysfunctions to, less frequently, a clear fronto-temporal dementia. An increasing need for reliable assessment of cognitive impairments in ALS is not easily met by regular psychometric methods due to their dependence on patients' unimpaired motor function. One of the recently considered motor-free alternatives in the assessment of cognitive impairments is the observation of changes in neuro-correlates of cognitive processes such as late event-related potentials (ERPs). While existing ERP studies of ALS have indeed identified changes in cognition-related ERPs, they have so far failed to identify sensitive markers of cognitive dysfunction, leading the authors to question the utility of ERP measurements in clinical ALS evaluation. Most of the studies were however limited to single or multiple channel waveform analysis and have not made full use of the topographical information provided by dense-array EEG recording. The aim of this study was to explore possible electrophysiological markers of cognitive change in non-demented patients with early-stage sporadic ALS using topographical ERP analysis of dense-array EEG recordings.

Methods: We recruited 12 patients with sporadic ALS (9 with limb-onset, 3 with bulbar-onset), all fulfilling the criteria of a 'probable or definite' ALS according to international classification of El Escorial – Revised and 11 healthy control subjects. We used a classical visual two-stimulus 'oddball' counting paradigm to evoke P3 ERP. Subjects' EEG was recorded with a 128-channel EEG (Brain Products actiCAP) recording system according to the International 10-5 System.

Each participant performed at least 4 blocks of trials comprised of 100 stimuli each, counting mentally the number of targets and reporting it at the end of each block. The Spherical Spline Laplacian (SSL) method was used to estimate average cortical surface potentials in target condition for each subject. A template response was then computed based on the control group grand-average, and both groups were compared on how well they matched the template in ERP amplitude, topography and time-course.

Results: Comparison of patient and control groups showed marked differences in all three ERP analysis domains: amplitude, topography and latency. Using binary logistic regression, topography and time-course data provided near perfect prediction of group membership.

Conclusion: Though preliminary, our findings indicate the presence of robust differences in late cognition-related ERPs in non-demented, sporadic ALS patients. Not relying on intact motor function, cognitive ERPs may provide a fruitful alternative to classical psycho-metric methods for testing cognition in ALS patients. Current results indicate that it might be possible to further develop this ERP method for the purposes of diagnosing and monitoring the progression of the disease.

P204 MOVEMENT-RELATED CORTICAL POTENTIALS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

BIZOVICAR N, ZIDAR I, KORITNIK B, ZIDAR J

Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia

E-mail address for correspondence: natasa.bizovicar@gmail.com

Keywords: movement related cortical potential, functional tests, respiration

Background: Respiratory failure is the main cause of death in patients with ALS. It is not known whether upper motor neuron loss contributes to it. Sniff nasal inspiratory pressure is a reliable marker of respiratory insufficiency in ALS. Therefore we have chosen sniffing to study cortical neural correlates of voluntary breathing. Each voluntary movement is preceded by a movement-related cortical potential (MRCP) that can be recorded from the scalp. It reflects the activity of the primary and secondary motor cortical areas. When associated with sniffing, it is called sniffing-related cortical potential (SRCP).

Objectives: Our aim was to study MRCPs related to finger flexion and sniffing as possible neural markers of the upper motor neurone dysfunction of hand and respiratory muscles. We searched for correlations between the SRCP amplitudes and the results of tests that measure respiratory function and also between the MRCP amplitudes obtained by finger flexion and the hand function tests.

Methods: Fourteen ALS patients (aged 42-77 years) and 15 healthy volunteers (aged 45-71 years) were studied. None of them had symptoms or clinical signs of respiratory insufficiency. They were assessed for their hand dexterity and strength, respiratory function, speech capacity, and upper motor neuron burden, Ashworth, Norris, and ALS functional rating scales. Electromyographic parameters used were neurophysiological index and the diaphragm CMAPs. Subjects performed self-paced sniffing every 5-10s with 20% of maximal sniff inspiratory pressure and the right finger flexion every 5-10s with 20% of maximal finger flexion pressure. The nasal pressure and the pressure produced by finger flexion were used as signals for back averaging of the EEG (10-10 system, 32 electrodes). The SRCP and MRCP amplitudes detected by electrodes Cz, C3, C4, FC5 and FC6 were measured.

Results: No significant differences of SRCP and MRCP amplitudes were found between the patients and healthy control subjects. In patients, there was a significant positive correlation between the SRCP amplitudes and scores of the Ashworth and bulbar part of the upper motor neuron burden scales, and oxygen saturation. Significant negative correlation was found between the SRCP amplitude and scores of the Norris scale. Significant negative correlation was also found between the MRCP amplitude and scores of the Ashworth scale and the time needed to perform the nine-hole peg test. There was a positive correlation between the MRCP amplitude and the results of the Tapping Board test.

Conclusions: Patients as a group did not have smaller amplitudes of the cortical potentials. Amplitudes of SRCPs were mostly not related to respiratory function tests. Higher amplitudes of finger-flexion MRCPs were related to better hand function. This finding maybe offers a possibility of using the MRCPs as biomarkers of disease severity.

P205 UPPER TRAPEZIUS ELECTROMYOGRAPHY AIDS EARLY DIAGNOSIS OF BULBAR INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS

XU Y, ZHENG J, ZHANG S, ZHANG J, FAN D

Peking University Third Hospital, Beijing, China

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: electromyography, trapezius, bulbar involvement

Background and objectives: Electromyography (EMG), particularly tongue or sternocleidomastoid EMG, aids in amyotrophic lateral sclerosis (ALS) diagnosis and can be used to identify lower motor neuron lesions in the bulbar region. Abnormal trapezius EMG was recently shown to be useful in ALS diagnosis. Here we investigated the role of upper trapezius EMG in assessing bulbar involvement in ALS.

Methods: A standard EMG was recorded from the upper trapezius in ALS patients, cervical spondylotic myelopathy (CSM) patients, and normal controls. Forty-three CSM patients were examined pre-operation and three months post-operation.

Results: Greater spontaneous activity levels were seen in the upper trapezius EMG of ALS patients with disease durations of ≤ 8 months (70%) than in patients with durations of > 8 months (40%). Significant differences in the motor unit action potential parameters were noted between ALS patients and normal controls or CSM patients. Fewer spontaneous activities were detected post-operation in CSM patients. No differences in neurogenic EMG changes were observed between the trapezius and sternocleidomastoid in ALS patients.

Conclusions: Upper trapezius EMGs may provide valuable information for assessing the clinical and subclinical involvement of bulbar lower motor neurons in ALS patients, particularly at early disease stages.

P206 STERNOCLEIDOMASTOID AND SCALENUS ANTERIOR MUSCLES INVESTIGATION IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: AN ELECTROMYOGRAPHIC STUDY

DE CARVALHO M[1,2], PINTO S[2], SWASH M[3,2]

[1]*Department of Neurosciences, Hospital de Santa Maria, Lisbon, Portugal,* [2]*Neuromuscular Unit, Instituto de Medicina Molecular, Lisbon, Portugal,* [3]*Department of Neurology, Royal London Hospital, Queen Mary School of Medicine, University of London, London, United Kingdom*

E-mail address for correspondence: mamedemg@mail.telepac.pt

Keywords: electromyography, diagnosis, monitoring

Background: To confirm diagnosis of ALS on electromyography (EMG) it is important to disclose abnormal findings in cranial innervated muscles. It is surprising that in patients with weak neck flexion the Sternocleidomastoid (SCM) muscle is frequently normal on EMG.

Objectives: We aimed to test SCM and scalenus anterior (SA) in ALS to test sensitivity of EMG applying different new techniques.

Methods: SM and SA were tested in 49 and 38 controls, respectively. SA needle placement was confirmed by ultrasound in 2 controls. In this population interferential pattern analysis of the EMG signal was performed, obtaining: number of turns (change in the motor unit potential polarity in the same phase), mean amplitude (amplitude of turns), turns/amplitude ratio, number of short-segments (number of intervals between successive turns); activity (percentage of time with EMG activity) and enveloped amplitude (amplitude without outliers). In addition in 25 (SCM) and 20 controls (SA) the new method of peak-ratio (PR) was calculated (peak-ratio and number of short-segments). The same approach was performed in 60 ALS patients (PR in a subset of 37 patients). Both groups were matched for sex and age. All ALS patients had probable or definite disease, with ALS-FRS $>$ 25 and neck flexor strength $>$ 3 (MRC) and 27 had bulbar-onset disease. A P value $<$ 0.01 was taken as significant.

Results: Comparing controls vs ALS, for both SCM and SA, number of turns, turns/amplitude, activity, number of short-segments and PR showed differences. In SCM, 42% of ALS patients had at least one abnormal turn-amplitude parameter, and in SA 67% showed this abnormality ($P>0.01$). For PR, this number was 27% and 72% for SCM and SA, respectively ($P<0.001$). The most abnormal turn-amplitude parameter was number of short-segments for both SCM (25%) and SA (53%). In SCM and SA we did not find a difference in the frequency of abnormalities between bulbar and spinal-onset patients.

Discussion and conclusions: Both turn-amplitude analysis and PR are easy to perform in neck muscles and disclose a large number of abnormalities in early-affected ALS patients. The sensitivity is similar between both methods. The SA shows more changes than the SCM muscle, supporting the observation of normal SCM on EMG in patients with weak neck flexion. Neither muscle is particularly more sensitive in bulbar-onset patients. SA is useful in the EMG assessment of a diagnosis of ALS.

P207 METABOLIC DYSREGULATION OF LIPID, URATE AND IRON IN SERA OF PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS: CORRELATION BETWEEN SEROLOGICAL CHANGES AND DISEASE PROGRESSION

IKEDA K, YOSHII Y, KAWABE K, ISHIKAWA Y, IWAMOTO K, IWASAKI Y

Toho University Omori Medical Center, Tokyo, Japan

E-mail address for correspondence: keni@med.toho-u.ac.jp

Keywords: lipids, urate, ferritin

Background: Several pathogenetic and prognostic factors are speculated in familial and sporadic forms of amyotrophic lateral sclerosis (ALS). Previous reports suggested distinct profiles of serum lipid, urate and ferritin levels in ALS patients. Dyslipidemia and hyperuricemia are protective markers and suggest a favorable prognosis in ALS patients.

Objectives: The purpose of this study is to examine serological changes of lipid, urate, and iron metabolites in Japanese patients with sporadic ALS. We also analyzed whether those serum levels were linked to disease course.

Methods: Seventy-nine patients with definite or probable ALS fulfilling the El Escorial revised criteria were analyzed for age, sex, limbs or bulbar onset, disease duration, ALS Functional Rating Scale (FRS), body mass index (BMI) and forced vital capacity (FVC), and serum levels of the following 9 items; total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, urate, iron, transferrin, ferritin and creatinine. Those variables were measured twice at the initial examination and one year later. All patients had neither family history of ALS nor mutations of superoxide dismutase (SOD) 1. ALS patients who had hypertension, renal dysfunction, gout, chronic inflammatory diseases, a percutaneous enterogastrostomy, iron medication, and UA- and lipid-lowering prescription were excluded from this study. Serological variables were compared between ALS patients and 80 healthy controls matotal cholesterolhed by age, sex and BMI.

Results: As compared to controls, serum urate and creatinine levels were decreased significantly in male and female patients with ALS. Serum ferritin levels were increased significantly in men and women with ALS. A significant reduction of serum high density lipoprotein cholesterol levels, and significant elevations of serum total cholesterol and triglyceride levels were found in female ALS patients. No statistical differences of total cholesterol, low density lipoprotein, high density lipoprotein and triglyceride existed between sera of male ALS patients and controls. Serum iron and transferrin levels did not differ between male and female ALS and controls. Impairment of FRS and FVC were delayed significantly in ALS patients with serum total cholesterol levels $\geq$ 200 mg/dL or low density lipoprotein $\geq$ 130 mg/dL at baseline. Annual decline rate of FRS was correlated with decline rate of serum creatinine levels, BMI and FVC. Baseline data of high density lipoprotein cholesterol, triglyceride, urate, iron, transferrin and ferritin did not influence decline rate of FRS.

Discussion and conclusions: The present study indicated significant decrease of urate levels and increase of ferritin levels in sera of ALS patients. Those serum levels were not associated with disease progression, including FRS, FVC and BMI. Higher serum total cholesterol and low density lipoprotein-C levels suggested favourable prognosis of ALS patients. Metabolic alternation of lipid, urate and ferritin could contribute to the pathogenesis and the progression of patients with sporadic ALS without SOD1 mutations.

P208 NO CORRELATION OF GANGLIOSIDE ANTIBODIES WITH DISEASE PHENOTYPE AND SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

KOLLEWE K, SINZENICH T, MOHAMMADI B, DENGLER R, WURSTER U, PETRI S

Medical School Hannover, Hannover, Germany

E-mail address for correspondence: kollewe.katja@mh-hannover.de

Keywords: ganglioside antibodies, phenotype

Introduction: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder with typical onset in the fifth- sixth decade of life. The earlier hypothesis of an autoimmune origin of ALS receives less attention today, but immunological phenomena still seem to be involved and mechanisms such as protective autoimmunity may be important. Antibodies against a variety of gangliosides also occur in ALS, but widely differing frequencies and titers have been reported. We investigated the presence of IgG and IgM antibodies in ALS patients.

Methods: IgG and IgM antibodies to the six gangliosides asialo-GM1 (GA1), GM1, GM2, GD1a, GD1b, GQ1b were determined by GanglioCombi ELISA in sera of 84 ALS patients with a mean age of 58. Results were expressed as a % ratio of a highly positive GM1 control and categorized as negative (<30%), borderline (30-50%), moderately (50-100%) and strongly positive (>100%). The values obtained from 220 Swiss blood donors (mean age: 52) served as a reference group.

Results: Twenty-two (26.2%) out of 84 ALS-patients showed elevated ganglioside antibodies: Taking all subspecific antibodies together, IgG antibodies were found in 9/84 (10.7%) and IgM in 15/84 (17.9%) patients. As 2 patients exhibited both isotypes, the combined frequency is 22/84 (26.2%). Three simultaneous antibodies occurred in 1 patient each for IgG (GA1, GD1a, GQ1b) and IgM (GM1, GD1a, GD1b). However, the frequencies for the individual antibodies were rather low, with maximal 7.1% (6/84) for IgG GD1a and 8.3% (7/84) for IgM GA1. Strongly positive values were found in 4 patients, 2 for IgG (GA1, GD1a) and 2 for IgM (GA1 and GM1). There was no statistically significant difference to the collective of normal blood donors.

Conclusions: Several studies with controversial results have assessed a potential association between elevated ganglioside-antibody-titres in ALS patients and specific disease phenotypes. Whilst in the existing literature mainly GM1- ganglioside antibodies were analysed, we used a novel assay to detect six subspecific IgM and IgG antibodies**.** Even with this more thorough approach, ganglioside antibody frequencies and patterns in our ALS cohort closely resemble the values observed in healthy controls, and the presence of ganglioside antibody was not correlated with age, gender, ALS phenotype or survival.

P209 ELIMINATING RATE IN LACTATE STRESS TEST: A POTENTIAL PREDICTOR FOR ALS SURVIVAL

ZHANG Y, FAN D

Peking University Third Hospital, Beijing, China

E-mail address for correspondence: dsfan@yahoo.cn

Keywords: lactate stress test, eliminating rate, progression

Objectives: Mitochondrial dysfunction plays an important role in the pathogenesis of amyotrophic lateral sclerosis (ALS). In the present study, we aimed to prove mitochondrial dysfunction in ALS by lactate stress test, and explore a possible association between lactate levels and clinical characteristics of ALS.

Methods: We carried out a cross-sectional study in 116 consecutive ALS patients. A standard procedure was performed: ALS patient rested completely for at least 15 minutes. After rest immediately before the test, lactate was determined for the first time (*lactate before*). Afterwards the patient was told to cycle with continuously close to uniform effort for 15 minutes, rotation speed 20-40 r/min, by a paddle-rate-independent electronic bicycle ergometer. Immediately after exercise a blood sample was acquired for lactate test (*Lactate 0*). Finally, after a rest for 15 minutes lactate was determined again (*lactate 15*). Plasma lactate concentrations were determined by high-performance liquid chromatography with fluorescence detection, whereas pyruvate at every time-point was determined also according to established guidelines. We calculated five deuterogenic values to seek an appropriate index, which are: (1)*Eliminating rate (ER)*=(*lactate15- Lactate0*)/15; (2)(*Lactate15*/*Lactate before*) ratio; (3)(*Lactate0*/*Lactate before*) ratio; (4)(*Lactate 0*/*Pyruvate Before*) ratio; (5)(*Lactate before*/*Pyruvate before*) ratio.

Results: We found a trend for patients with rapid slope of ALSFRS-r (>20 U/year) to have a slowest ER (median -4.67×10^{-3}mmol/L/min, Coefficient of variation, 590.15%), shortest duration (0.63±0.28 years) and worst ALSFRS-r (32.59±4.93). The patients with moderate slope of ALSFRS-r (10-20 U/year) have a moderate ER (median -11.33×10^{-3}mmol/L/min, Coefficient of variation, 309.89%), duration (1.16±0.45 years) and ALSFRS-r (34.83±6.11). The slower progression (<10 U/year) patients have a rapid ER (median: -12.00×10^{-3}mmol/L/min, coefficient of variation: 143.08%), longer duration (median: 3 years, coefficient of variation: 193.33%) and good ALSFRS-r value (39.58±9.44). Furthermore, we found advanced phase ALS patients (defined as definite and probable ALS) also have slower ER (quariles -17.33, -5.67, 4) and worse ALSFRS-r (34.88±9.27), while early phase patients (defined as laboratory supported probable and possible ALS) have more rapid ER (quariles -25.17, -11.33, -3.50) and better ALSFRS-r (39.28±7.59). All the differences are statistically significant. Multiple linear regression revealed a strong direct association between ER, ALSFRS-r slope (standard Beta=0.33, P=0.007) and FVC (standard Beta= -0.458, P=0.006, adjusted for ALSFRS-r, course and onset region).

Conclusions: This study was a cross-sectional design of clinical variables and laboratory numeric values, which prevented the assessment of a temporal relationship between decreased ER and motor decay. Our results suggested that slower ER may be linked to faster progression of the disease and has potential to be a biomarker for ALS survival.

P210 PATHOLOGICAL CHANGES IN THE LATERAL CORTICOSPINAL TRACT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS ARE ASSOCIATED WITH ALTERED NEUREGULIN EXPRESSION

SONG F, CHIANG P, LOEB J

Hiller ALS Clinic and Research Center, Department of Neurology, Center of Molecular Medicine and Genetics, Wayne State University, Detroit, MI, United States

E-mail address for correspondence: fsong@med.wayne.edu

Keywords: neuregulin, lateral corticospinal tract, biomarker

Background: Amyotrophic lateral sclerosis (ALS) is poorly understood at the molecular level and there are as yet no

effective therapeutics to stop the insidious progression that involves both the upper and lower motor systems. ALS mainly affects lower motor neurons in the spinal cord that are functionally related to upper motor neurons in the cortex through monosynaptic connections (1). Surprisingly even less is known about the cause of pathological progression within the lateral corticospinal tract (LCST) that connects the upper and lower motor systems. These monosynaptic interactions between upper and lower motor neurons are not affected in the most commonly studied mouse and rat models (2), leaving only studies on postmortem human tissues to begin to understand this region of progressive degeneration in ALS.

Objectives: Here we have set out to identify biomarkers as well as potential drug targets from postmortem human ALS patients with special attention to the pathological changes in the LCST.

Methods: Pathological changes were measured for motor neuron number, axon loss, demyelination, gliosis and microglial activation in both patients with ALS and controls. Gene expression and protein levels were determined by quantitative PCR for a number of genes implicated in the disease process as well as the NRG1 gene isoforms that have important roles in nervous system development.

Results: The spinal cord and brain of patients with ALS showed varying degrees of neuronal loss and activation of microglial and astrocytes both within the ventral horn and LCST. While there was no consistent change in SOD1, TDP43, and GLT1 (genes implicated in pathogenesis), there was a significant downregulation of NRG1 gene expression (a neuronally-expressed gliotrophic gene that supports axoglial interactions) in the spinal cord but slight upregulation in the brain. Up-regulation of type III NRG1 protein expression was observed both in the brain and the LCST and was associated with axonal loss and demyelination in the LCST and with microglial activation/infiltration. The down-regulation of type III NRG1 protein in spinal motor neurons is also microglial activation in the ventral grey matter.

Conclusions: Type III NRG1, an important glial regulatory factor produced by neuronal axons, is dysregulated in both upper and lower motor neurons in patients with ALS in both the ventral horn and LCST.

Discussion: Altered expression of this important growth and differentiation factor could result in altered cell-cell signal patterns in ALS that could both lead to disease progression as well as serve as a biomarker for neuronal loss and pathological changes within the LCST.

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P211 A HIGH THROUGHPUT IMAGE ANALYSIS STRATEGY FOR ANALYSIS OF *IN SITU* HYBRIDIZATION TRANSCRIPT PATTERNS IN THE SOD1_G93A MOUSE MODEL OF ALS

LINCECUM J[1], WANG M[1], PUCHALSKI R[1], SANCHEZ R[1], CARRION I[1], VIEIRA F[1], PERRIN S[1]

[1]*ALS TDI, Cambridge, MA, United States,* [2]*Allen Institute for Brain Science, Seattle, WA, United States*

E-mail address for correspondence: jlincecum@als.net

Keywords: ISH, SOD1_G93A, algorithm

The recent acceleration of the pace of high throughput genomics and the generation of complex gene networks has underscored the need for technologies capable of capturing and quantifying gene expression patterns in tissues. The traditional method for visualizing patterns of gene expression is *in situ* hybridization (ISH). ISH originally employed radioactive probes, manual hybridization to processed tissue sections, and arduous autoradiography to see patterns of gene expression. This radioactive method has since been improved using 'cold' techniques based on hapten labeled riboprobes and immunohistochemical detection of the signal. Optimized methods for automation of tissue and slide processing with high throughput imaging have made possible large scale projects such as the Allen Institute for Brain Science's Mouse Brain Atlas and Mouse Spinal Cord Atlas projects (www.brain-map.org). Working in collaboration with the Allen Institute we have generated a database of several thousand transcript patterns covering the presymptomatic and early symptomatic spinal cord of SOD1_G93A mouse model of ALS.
Here we report on our efforts to implement an automated solution to the image analysis of these patterns of gene expression. Using a software package, CellProfiler 2.0 (1) we have developed an image analysis pipeline capable of quantifying patterns of transcript expression during disease progression in the SOD1_G93A spinal cord. The algorithm has been successfully applied to a panel of neuronal and inflammatory markers, such as GFAP and CD68, and shown to be robust and reproducible. In conclusion, automated image analysis of complex patterns of gene expression makes possible development of biomarker panels of cell state applicable to pre-clinical pharmacodynamic screening in this mouse model of ALS.

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P212 BIOMARKERS IN A TRANSGENIC MOUSE MODEL OF NEURODEGENERATION

CALVO A[1], TORRE-MERINO P[2], MANZANO R[1], OLIVÁN S[1], MUÑOZ JM[1], ZARAGOZA P[1], GARCÍA-REDONDO A[2], OSTA R[1]

[1]*LAGENBIO-I3A, Aragonese Institute of Health Sciences (IACS), Faculty of Veterinary Science, University of Zaragoza, Miguel Servet, Zaragoza, Spain,* [2]*Servicio de Neurología - Unidad de ELA, CIBERER U-723, Instituto de Investigación Sanitaria Hospital 12 de Octubre, Madrid, Spain*

E-mail address for correspondence: accalvo@unizar.es

Keywords: biomarker, muscle biopsy, muscle atrophy

Background: Amyotrophic Lateral Sclerosis (ALS), a progressive motor neuron disease, is one of the most common neurodegenerative disorders. ALS causes muscle atrophy together with a degeneration of motor neurons leading to death within 3 to 5 years after onset. Many studies have been carried out to detect molecular markers for diagnosis of ALS in different tissues like blood or cerebrospinal fluid. However,

the molecular targets that prompt the neurodegenerative process in ALS remain unknown.

Objectives: Our main goal is to study, throughout disease progression, the levels of expression of groups of genes directly related to the atrophy and regeneration of skeletal muscle, one of the tissues most affected by the disease, in order to find molecular biomarkers for ALS.

Methods: Inbred B6SJL $SOD1^{G93A}$ mice were used as they have been established as a suitable ALS disease model. Muscle biopsies were carried out in males and females mice at the asymptomatic, symptomatic and terminal stages. Biopsy samples were processed for total RNA extraction. Gene expression variations in all samples were assayed by real-time PCR. Genes related to muscle damage; muscle differentiation and regeneration; maintenance of muscle integrity and muscle reinnervation; calcium homeostasis; glucose homeostasis and oxidative stress were studied. Statistical analysis was carried out to find correlation between gene expression levels throughout disease progression and longevity.

Results: Genes involved in the maintenance of muscle integrity and in the inhibition of muscle reinnervation correlated with longevity in both males and females. Furthermore, genes involved in muscle damage, muscle differentiation and regeneration, glucose metabolism and calcium homeostasis were correlated to longevity only in females.

Discussion and conclusions: The different gene expression profile and correlations found in males and females suggest that the skeletal muscle behaves in a different way in both sexes under neurodegenerative conditions. Female transgenic mice seem to compensate for the activation of degeneration signals in skeletal muscle more efficiently than males, delaying muscle differentiation and buffering deregulation of calcium and glucose homeostasis. These results could shed light to find new biomarkers in different tissues in $SOD1^{G93A}$ mice and in a later step to translate the study of these biomarkers to human samples, so as to finally reach a more accurate knowledge of the disease.

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P213 DYNAMIC *IN VIVO* OPTICAL IMAGING AND MRI FOR TRACKING NANOPARTICLES AND EVALUATING DISEASE PROGRESSION IN SOD1(G93A) MICE

SHARP P, BERWICK J, KENNERLEY A, WANG L, MADSEN J, ARMES S, MEAD R, SHAW P, AZZOUZ M, BATTAGLIA G

The University of Sheffield, Sheffield, United Kingdom

E-mail address for correspondence: p.sharp@sheffield.ac.uk

Keywords: nanoparticles, MRI, optical imaging

Background: A major obstacle in the treatment of neurological disorders is the blood-brain-barrier (BBB), which limits the entry of many potentially therapeutic agents into the central nervous system (CNS). Advances in nanotechnology have led to the development of biocompatible nanoparticles, which can act as nanometer-sized vectors for therapeutic agents. One promising class of nanoparticle, known as polymersomes, are made of amphiphilic block copolymers that self-assemble to form polymeric vesicles. We have previously demonstrated efficient cytosolic delivery of active agents using polymer vesicles in numerous cell lines. Our aim now is to examine their application *in vivo* to effectively deliver neuroprotective agents into the CNS. To enhance BBB translocation, polymeric vesicles will be functionalized (ligand-decorated) to target receptor-mediated transcytosis at the BBB. Optimal formulations identified will be used to enhance delivery of therapeutic agents in SOD1(G93A) mice.

Objectives: To evaluate the potential of numerous polymersome formulations, we aim to develop a quantitative *in vivo* optical imaging technique to characterize the efficiency in which fluorescently labeled polymersomes can traverse the BBB. Furthermore, in preparation for therapeutic testing in SOD1(G93A) mice, we also aim to establish the use of high-field MRI to precisely measure key pathological markers of disease.

Methods: Cerebral vasculature was visualized through a cranial window of terminally anaesthetized rats. Rhodmine labeled polymersomes were administered intravenously and fluorescent signal was measured in the vascular and extravascular compartments. For the longitudinal MRI study, SOD1(G93A) transgenic mice (n = 5) and non-transgenic littermates (n = 5) were scanned every 30 days throughout their life span.

Results: Dynamic image analysis of cerebral vessels *in vivo* showed that the fluorescent signal generated by rhodamine labeled polymersomes was readily detectable and stable. In contrast, signal generated by free rhodamine was highly transient, demonstrating the stability of polymersomes in circulation. The polymersomes examined were not functionalized, so no increase in signal in extravascular regions was detected. MRI scans showed that hindlimb and pelvic muscle volumes of SOD1(G93A) transgenic mice were significantly smaller than controls as early as 30 days of age, which is almost 50 days prior to the onset of overt behavioural signs. Subsequent analysis showed a considerable difference between transgenic and control mice at 60, 90, and 120 days of age. In addition, clearly defined lesions were also detected in the brainstem of transgenic mice at 90 and 120 days of age.

Discussion and conclusions: We report here a novel optical imaging based methodology for sensitive monitoring of fluorescently labeled nanoparticles *in vivo*. This approach will be invaluable for assessing BBB penetration of nanoparticle-based platforms. We also demonstrate that MRI is a powerful imaging tool for tracking pathological changes in SOD1(G93A) mice, which will be applied in future preclinical efficacy studies.

P214 PROGRESSIVE CHANGES IN SPINAL MOTOR SYSTEM IN AN ADULT MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

JIANG M, SCHUSTER J, FU R, SIDDIQUE T, HECKMAN CJ

Northwestern University, Chicago, Illoise, United States

E-mail address for correspondence: m-jiang@northwestern.edu

Keywords: root recording, interneurons, bursts

Background: Amyotrophic lateral sclerosis (ALS) is a late onset motor disease characterized by progressive degeneration of motoneurons. Excitotoxicity is one of several mechanisms being investigated for motoneuron degeneration in ALS. It is generally considered that the excitotoxicity arises from excessive Ca^{2+} entry into motoneurons and the poor motoneuronal Ca^{2+} buffering. The excessive Ca^{2+} entry may occur when excitatory synaptic inputs or voltage-sensitive Ca^{2+} channels

become more active, resulting in increased opening of either Ca^{2+} permeable AMPA and NMDA receptors or voltage-sensitive Ca^{2+} channels. On the other hand, decrease in inhibitory synaptic transmission may disinhibit motoneurons and cause increase of Ca^{2+} entry. Thus, identification of detailed excitotoxic source is critical for its pathogenesis and therapy.

Objectives: The objective of this investigation was to evaluate the peripheral monosynaptic inputs and spinal pre-motor network that may contribute to the excitotoxicity of spinal motoneurons of mSOD1 mice.

Methods: Under urethane anesthesia, spinal cords containing sacral 1-3 segments (S1-3) and their ventral and dorsal roots were taken from $mSOD1^{G93A}$ mice and their nontransgenic littermates (NG) at three age groups (P50, P90 and > P120), and mounted in a recording chamber superfused with artificial brain-spinal fluid. The dorsal roots and distal ends of ventral roots were placed on stimulating electrodes while the proximal ventral roots were placed on recording electrodes. Chemicals were added to spinal cord through bath application. The evoked root responses were compared between mSOD1 and NG mice. To test spinal interneurons, strychnine, the glycine receptor antagonist, and picrotoxin, the GABAa receptor antagonist, were applied to generate synchronized bursts. Short train stimulation (five pulses) was also delivered to the dorsal root to determine the role of inhibitory synapses in ALS.

Results: The ventral root responses evoked by both peripheral and ventral root stimulation progressively decreased with age, making the peripheral monosynaptic input unlikely as an excitotoxic source. After symptom onset, a higher percentage of mSOD1 mice exhibited bursting with more subbursts and increased randomness. In mSOD1 mice with clear muscle tremor, the ventral roots exhibited spontaneous synchronized bursts, which were highly sensitive to NMDA receptor blockade. It was also demonstrated that inhibitory synapses participate in short-term depression (STD) at peripheral-motoneuron synapses.

Discussion: Although the induced and spontaneous bursts in mSOD1 mice indicate a role of interneurons in ALS pathogenesis, the involvement of receptors requires further investigation. By testing STD, this continuing study examines the functional status of GABAa/glycine receptors between mSOD1 and NG mice.

Conclusion: The data suggest progressive increase in excitability of spinal interneuons in mSOD1 mice that may lead to an excitotoxic effect on motoneruons.

P215 THE EFFECT OF THE ELECTRICAL AND MORPHOLOGICAL ALTERATIONS IN MUTANT SOD1 MOTONEURONS ON THE DENDRITIC PROCESSING OF SYNAPTIC INPUTS

ELBASIOUNY S[1], DURAND J[2], HECKMAN CJ[1]

[1]*Northwestern University, Chicago, IL, United States,* [2]*CNRS, Université de la Méditerranée, Marseille, France*

E-mail address for correspondence: s-elbasiouny@northwestern.edu

Keywords: electrophysiology, modelling, excitotoxicity

Background: Mutant SOD1 (mSOD1) motoneurons in the standard G85R mouse model of ALS exhibit alterations in morphology (1) and electrical properties (2,3), long before symptom onset (P8-P10). These alterations are expected to affect the motoneuronal processing of synaptic inputs and consequently motoneuron recruitment during movement.

Objectives: To assess the individual roles that the changes in morphology, electrical properties, and dendritic ion channels play in the efficacy and summation of synaptic currents in wild-type (WT) and mSOD1 motoneurons.

Methods: We used realistic computer models of WT and mSOD1 lumbar motoneurons in which the motoneuronal electrical and morphological properties could be manipulated independently. Models were based on the reconstructed morphologies of neonatal WT and mSOD1 motoneurons (P8-P10) (1), and model parameters were optimized to match the electrophysiological properties recorded experimentally from the same motoneurons.

Results: Our results from computer simulations indicated that: First, the change in morphological properties does not account fully for the change in electrical properties. Second, the specific membrane resistance (Rm) was reduced in mSOD1 motoneurons by 25% relative to WT. Third, one-third of the reduction in mSOD1 motoneurons input resistance (Rin) relative to WT (2), is due to the morphology change, whereas the other two-thirds are due to the reduction in Rm. Fourth, synaptic efficacy is reduced in mSOD1 motoneurons by 25% (ie -25%), relative to WT. This total change resulted from the concurrent increase in morphology, reduction in Rm, and the increase in dendritic voltage-gated ion channels, which individually accounted for -10% to -15%, -23%, and +25% change in synaptic efficacy, respectively. Finally, the summation of synaptic currents in mSOD1 motoneurons did not exhibit significant differences from that in WT motoneurons despite the alteration in morphology, electrical properties, and synaptic efficacy.

Discussion and conclusions: Our results provide a quantitative analysis of the early alterations in morphology and electrical properties of mSOD1 motoneurons and illustrate the functional consequences of those alterations on the dendritic processing of synaptic inputs. The early reductions in Rin and synaptic efficacy of mSOD1 motoneurons would hinder their recruitment, and could contribute to the later retraction of axons from the neuromuscular junction. This prediction is consistent with the beneficial effects of increased neuromuscular activity that improved motoneuron survival in ALS (4). Our simulations also predict changes in the biophysical properties of mSOD1 motoneurons, and set the stage for new experiments to test these predictions.

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P216 MOTOR UNIT NUMBER ESTIMATION USING THE INCREMENTAL METHOD IN NORMAL DOGS

VASQUEZ L, KANAZONO S, VILLAMIL AJ, DHAND U, O'BRIEN D, COATES J

University of Missouri, Columbia, MO, United States

E-mail address for correspondence: vasquezl@missouri.edu

Keywords: canine, degenerative myelopathy, MUNE

Background: Canine degenerative myelopathy (DM) is an adult onset fatal neurodegenerative disease. Initial upper motor neuron paraparesis and general proprioceptive ataxia progress to lower motor neuron tetraparesis. Recently a missense mutation in the canine superoxide dismutase 1 (*SOD1*) gene has been shown to be a risk factor for DM suggesting homology to human familial amyotrophic lateral sclerosis (ALS) (1). Motor unit number estimation (MUNE) is a technique for quantifying motor unit function and assessing disease progression in ALS patients. If the technique can reliably be established in normal dogs, MUNE could be used as an outcome measure for monitoring therapies in DM affected dogs.

Objective: To adapt the incremental method for MUNE in normal healthy dogs and establish a reference range.

Methods: The incremental stimulation technique described in humans was modified and used on the extensor digitorum brevis (EDB) muscles of 17 normal dogs (age: 1-10 years; body weight 4.5-70 kg) (2). Monopolar stimulating needle electrodes were inserted caudal to the long digital extensor muscle tendon at the level of the tuber calcanei to stimulate the deep peroneal nerve. Direct evoked muscle potentials were recorded with the active surface electrode placed over the EDB motor point and the reference surface electrode over digit IV. The ground electrode was placed subcutaneously between cathode and recording electrode. Supramaximal compound muscle action potential (CMAP) negative peak area was first recorded. Then starting at the subthreshold level, stimulus intensity was slowly increased until the first all-or-none single motor unit potential (SMUP) was evoked. Successive small increments (0.026 mA, 50 µsec) were applied to elicit a total of 10 SMUP responses. The mean SMUP negative peak area was divided into the maximum CMAP negative peak area to yield the MUNE value. Multiple trials were performed on both hindlimbs to assess reproducibility.

Results: The median CMAP area was 4.95 mV·mS (range 1.89-9.32) with 25 and 75 percentile of 3.72 and 5.98, respectively. The median SMUP area was 0.10 mV·mS (range 0.03-0.81) with a 25 and 75 percentile of 0.07 and 0.16, respectively. The MUNE median was 49 (range 8-154) with 25 and 75 percentile values of 30 and 68, respectively. There was no significant difference in MUNE between age groups older and younger than 7 years ($P=0.17$) or between right and left limbs ($P=0.14$).

Discussions and conclusions: We demonstrated that EDB incremental method MUNE can be reliably recorded from normal dogs. These results provide potential to apply the described technique for longitudinal monitoring of lower motor neuron signs in DM affected dogs.

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P217 CEREBROSPINAL FLUID NEUROTRANSMITTER CONCENTRATIONS IN CANINE DEGENERATIVE MYELOPATHY; FURTHER SUPPORT OF A CANINE MODEL OF UMN ONSET ALS

PLATT S[1], BARBER R[1], KANAZONO S[2], EDWARDS G[1], COATES J[2]

[1]*College of Veterinary Medicine, University of Georgia, Athens, GA, United States,* [2]*College of Veterinary Medicine, University of Missouri, Columbia, MO, United States*

E-mail address for correspondence: srplatt@uga.edu

Keywords: canine, degenerative myelopathy, neurotransmitters

Background: Recently a missense mutation in the canine superoxide dismutase 1 (*SOD1*) gene has been shown to be a risk factor for DM suggesting homology to human familial amyotrophic lateral sclerosis (ALS) of UMN onset. Investigations of cerebrospinal fluid (CSF) neurotransmitter concentrations as part of the pathogenesis in human ALS patients suggest a role for excitotoxicity. Treatment strategies for excitotoxicity could be investigated in DM affected dogs if a similar pathogenic role can be suggested.

Objectives: To determine presence or absence of abnormalities in the concentrations of CSF amino acid neurotransmitters: glutamate, glycine and g-aminobutyric acid (GABA) in dogs with DM.

Methods: Forty-six dogs histopathologically confirmed for DM at different clinical stages and 41 clinically normal age-matched control dogs were included in the study. Cerebrospinal fluid was acquired from the cerebellomedullary cistern of dogs while under general anesthesia for diagnostic testing or immediately after euthanasia. All CSF samples were stored at -80°C until time of analysis; 100 µl was deproteinized with methanol (100 µl) and centrifuged at 14,000 g for 2 min. Deproteinized samples were then analysed for presence of glutamate, glycine and GABA by high performance liquid chromatography with fluorescence detection on an Agilent 1100 system following pre-column derivatization with o-phthaldialdehyde (OPA) and 3-mercaptopropionic acid (3-MPA). All analyses were performed using SAS V 9.2 (Cary, NC). Analyte levels were compared between DM affected and age matched control dogs by an analysis of variance (ANOVA). All hypothesis tests were 2-sided with $\alpha=0.05$.

Results: Glutamate levels were not significantly different between DM affected (mean=0.05 µg/ml; 0.0-0.29; SD=0.069) and control dogs (mean=0.04 µg/ml; 0.0-0.29; SD=0.077). Control dogs (mean=0.94 µg/ml; 0.78-1.16; SD=0.164) had significantly higher levels of GABA ($P<0.0001$;) than affected dogs (mean=0.10 µg/ml; 0.08-0.19; SD=0.03). Control dogs (mean=2.4 µg/ml; 1.24-5.18; SD=0.82) also had significantly higher glycine concentrations ($P<0.0001$) than affected dogs (mean=0.4 µg/ml; 0.01-0.78; SD=0.23).

Discussion and conclusions: We demonstrate that DM affected dogs have an imbalance of CSF amino acid concentrations creating a relative excitotoxic environment. Reports in human ALS describe that CSF glutamate is decreased with mild clinical signs, whereas in those with severe clinical signs glutamate will vary from decreased to increased. Similar divergent results have been reported for CSF GABA, glycine and aspartate. An explanation for such differences has been suggested to be to due to disease heterogeneity. Despite these contradictory reports it is believed that an imbalance between CSF excitatory and inhibitory amino acids plays a pathogenic role in ALS. Further prospective analysis of the canine model is warranted to investigate the amino acid variations and possible role as a biomarker at early and late disease stages.

P218 CEREBROSPINAL FLUID FROM PATIENTS WITH SPORADIC ALS – A POTENTIAL TOOL TO DELINEATE THE ETIOPATHOGENESIS OF THE SPORADIC FORM OF THE DISEASE

RAJU TR

National Institute of Mental Health and Neuro Sciences, Bangalore, Karnataka, India

E-mail address for correspondence: trraju.nimhans@gmail.com

Keywords: cerebrospinal fluid, animal model, motor behaviour

Background: The progress in understanding the pathogenesis of sporadic ALS (SALS) has been challenged to a great extent by the absence of appropriate animal models for the disease. Unlike familial ALS, wherein precise genetic mutations have been defined, the SALS form has not been associated to any specific factors.

Objectives: In an attempt to study the etiopathogenesis of SALS, the effect of CSF from SALS patients (ALS-CSF) was tested on motor neurons of the cortex and spinal cord.

Methods: Initially, primary rat embryonic spinal cord cultures were exposed to ALS-CSF and the cellular changes were assessed. Also, to exclude the confounding effects of glia in inducing the changes, we exposed NSC-34 motor neuron cell line to ALS-CSF. To simulate the changes in a more complex system, we intrathecally injected ALS-CSF into neonatal rat pups. Further to determine whether ALS-CSF could induce explicit alterations in motor behaviour and spiking properties of motor neurons, we intracerebroventricularly infused ALS-CSF in adult rats. Local field potentials were recorded from layer V motor cortex in an unrestrained rat. The deficits in motor behaviour were quantified using rotarod and grip strength testing.

Results: ALS-CSF induced degenerative changes in the motor neurons and also affected the astrocytes. Reduced number of Choline acetyl transferase positive spinal motor neurons with aberrant phosphorylation of neurofilaments and enhanced LDH activity were amongst the prominent observations. Ultrastructurally Golgi apparatus were fragmented and numerous small, discrete and unconnected stacks were distributed widely throughout the cytoplasm. Similar changes were reproduced in NSC-34 cells in addition to ubiqitinated protein aggregates and recruitment of endopalsmic reticulum stress responses. Reduced expression of trophic factors indicated an altered microenvironment of motor neurons. Interestingly the expression of sodium and potassium channels on spinal motor neurons was significantly diminished. In line with this, the local field potentials recorded from motor cortex of adult rats showed a bimodal effect on the relative power values. Apart from motor neurons the astrocytes were also affected. Reactive astrogliosis was accompanied by the transformation of astrocytes from flat to fibrous form. Also, the expression of glial glutamate transporter-1 was reduced. These cellular and molecular changes were substantiated by an overt motor behavioral deficit on rotarod and grip strength.

Discussion and conclusion: This is the first comprehensive study to report the effect of ALS-CSF on motor neurons at the molecular, cellular, physiological and behavioral levels. Most of our findings reported here are comparable to those seen in autopsy samples of SALS patients and also animal models of FALS. The degeneration of motor neurons is resultant of the dysfunction of several related mechanisms.

Author Index

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Subject Index

mnda
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BIOMEDICAL RESEARCH GRANTS 2011

The MND Association's vision is A World Free of MND. Realising this vision means investing more funds in research, developing strategic partnerships with the research community and research funding bodies and ensuring that advances in understanding and treating MND are communicated as quickly and effectively as possible.

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Natasha Rowe
Research Grants Administrator
Tel: + 44 (0)1604 611873
Fax: + 44 (0)1604 638289
Email: natasha.rowe@mndassociation.org
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The *MRC/MND Association Lady Edith Wolfson Fellowships* aim to support clinicians wishing to pursue research into the pathogenesis and treatment of motor neuron disease. Awards under this scheme are available at three levels:

- ***Clinical Research Training Fellowships:*** up to three years support is available for clinically qualified, active professionals to undertake specialised or further research training. Applicants wishing to conduct patient orientated Clinical Research Training Fellowships may apply for 4 years funding. The scheme is designed to accommodate the dual clinical-research training career path by allowing Fellows to spend up to 20% of their time on NHS sessions. It is awarded at the clinical pre-doctoral or entry level, although medically qualified applicants who obtained their PhD some time ago may also apply. Fellows are required to register for a research degree, normally a PhD, based on research undertaken during the Fellowship. Applications are considered twice yearly. ***The next deadline for submission of applications is 20 January 2011.***

- ***Clinician Scientist Fellowships:*** these awards are post-doctoral Fellowships providing up to four years support for laboratory-based studies. There is also a patient-oriented version of the scheme that provides up to five years support, which is intended for research requiring up to 40% of the Fellow's time to be spent in clinical work. At least 50% of this clinical work should be of direct relevance to the research project. Applications are considered annually. ***See MRC website for full details and 2011 deadline.***

- ***Senior Clinical Fellowships:*** support for up to five years is awarded to clinical researchers of exceptional ability. Applicants are expected to be proven independent researchers in a field of investigation relevant to MND, to be well-qualified for academic research and to demonstrate promise as future research leaders. Applications are considered annually. ***See MRC website for full details and 2011 deadline.***

The *Lady Edith Wolfson Fellowships* will be administered by the MRC, from whom additional information; application forms and confirmation of deadline dates can be obtained. Please see the MRC website (www.mrc.ac.uk/Careers/Fellowships) for details

If you wish to discuss your proposed research area in advance of submission, please contact Dr Brian Dickie at the MND Association (brian.dickie@mndassociation.org).

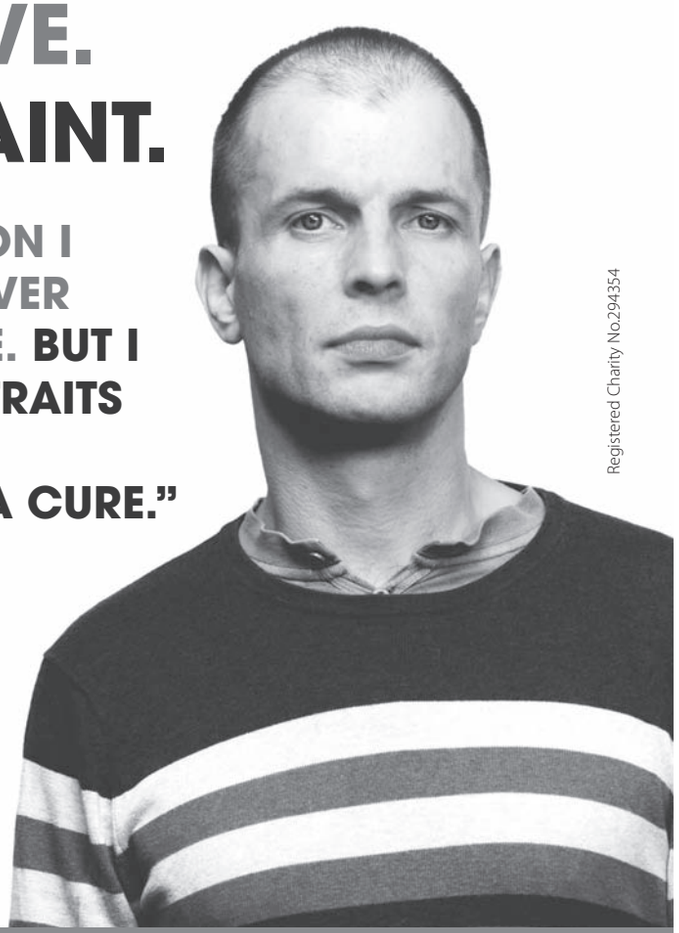
Keep up to date with deadline dates by following us on Twitter **@mndresearch**

Motor Neurone Disease Association, PO Box 246, Northampton NN1 2PR. Registered Charity No. 294354

Mint on Aegean/Research/Research only/Grants/MRC MND Association Fellowship flyer 2010

ONLY MONTHS TO LIVE.
100 PORTRAITS TO PAINT.

"I'M PATRICK AND I'M AN ARTIST. SOON I WILL LOSE THE ABILITY TO PAINT FOREVER THANKS TO MOTOR NEURONE DISEASE. BUT I AM DETERMINED TO CREATE 100 PORTRAITS IN WHAT TIME I'VE GOT LEFT, TO RAISE AWARENESS OF MND AND HELP FIND A CURE."



mnda
motor neurone disease
association

patricktheoptimist.org

Registered Charity No.294354

The ALS Association is the only non-profit organization fighting Lou Gehrig's Disease on every front. By leading the way in global research, providing assistance for people with ALS through a nationwide network of chapters, coordinating multidisciplinary care through certified clinical care centers, and fostering government partnerships, The Association builds hope and enhances quality of life while aggressively searching for new treatments and a cure.

CALL FOR RESEARCH ABSTRACTS
DUE JANUARY 2011

RESPIRATORY CLINICAL MANAGEMENT

Seeking to build an increasing evidence base for the Respiratory Management of ALS

Request for Abstracts (RFA)

The ALS Association Patient Services requests abstracts for research projects concerning the interventional *Respiratory* management of ALS patients. Abstracts should emphasize clinical care of ALS patients.

Investigators are encouraged to submit an abstract of 1-2 pages for respiratory management. Abstract requirements are described in the Clinical Management Research section of The ALS Association's web site.

The ALS Association will consider projects of 1-2 years with a total maximum award of $50,000.

Grant Schedule	Spring Cycle – 2011
Abstracts Due	**24th January 2011**
Full Application Due	**25th March 2011**
Award Announcement	**June 2011**
Funding Commences	**August 2011**

For more information or to be added to the mailing list
Contact Sharon Matland
smatland@alsa-national.org
Visit

Phone: (818) 587.2217 or 2219
Fax: (818) 880-9006
www.alsa.org

BIOMEDICAL RESEARCH

Building an ALS TRANSLATIONAL RESEARCH PIPELINE

Request for Abstracts (RFA)

The ALS Association Research INVESTIGATOR-INITIATED RESEARCH GRANT PROGRAM supports INNOVATIVE research of high scientific merit and relevance to amyotrophic lateral sclerosis (ALS), offering investigators awards in the following categories:

Multi-year Grants
The ALS Association will support research that is projected for periods of up to three (3) years. Funding for multi-year grants is committed for one (1) year only, with noncompetitive renewals conditioned upon results. These applications require strong preliminary data. Awards will be in the amount of up to $80,000 per year.

Starter Grants
One-year awards for NEW INVESTIGATORS ENTERING THE FIELD OF ALS. Alternatively, they can be PILOT STUDIES BY ALS INVESTIGATORS. These applications do not require strong preliminary data but must emphasize innovation, scientific merit, feasibility and relevance to ALS. The maximum amount awarded is $40,000.

The Milton Safenowitz Post Doctoral Fellowship for ALS Research Awards (Spring cycle only)
The maximum amount awarded is $40,000 per year for 2 years. Eligibility is limited to those who have been a fellow for one year or less.

Request an abstract form for any of these categories from researchgrants@alsa-national.org. You will be notified within two weeks of the abstract submission due date whether you are eligible to submit a full application. See schedule below.

Grant Schedule	Spring Cycle - 2011
Call for Abstracts	**1st December 2011**
Abstracts Due	**5th January 2011**
Full Application Due	**4rd March 2011**
Award Announcements	**June 2011**
Funding Commences	**1st August 2011**

Phone: (203) 641-1158
E-mail: researchgrants@alsa-national.org

Award PG

3rd PG International Award - ALS/MND

Will be awarded on 30 November at the
22nd International Symposium on ALS/MND
30 November - 2 December 2011, Sydney Australia

Award for winner
US $20,000 + Gold Medal

Deadline for delivering papers
31st March 2011

Regulation and registrations at site
www.ipg.org.br/awardpg

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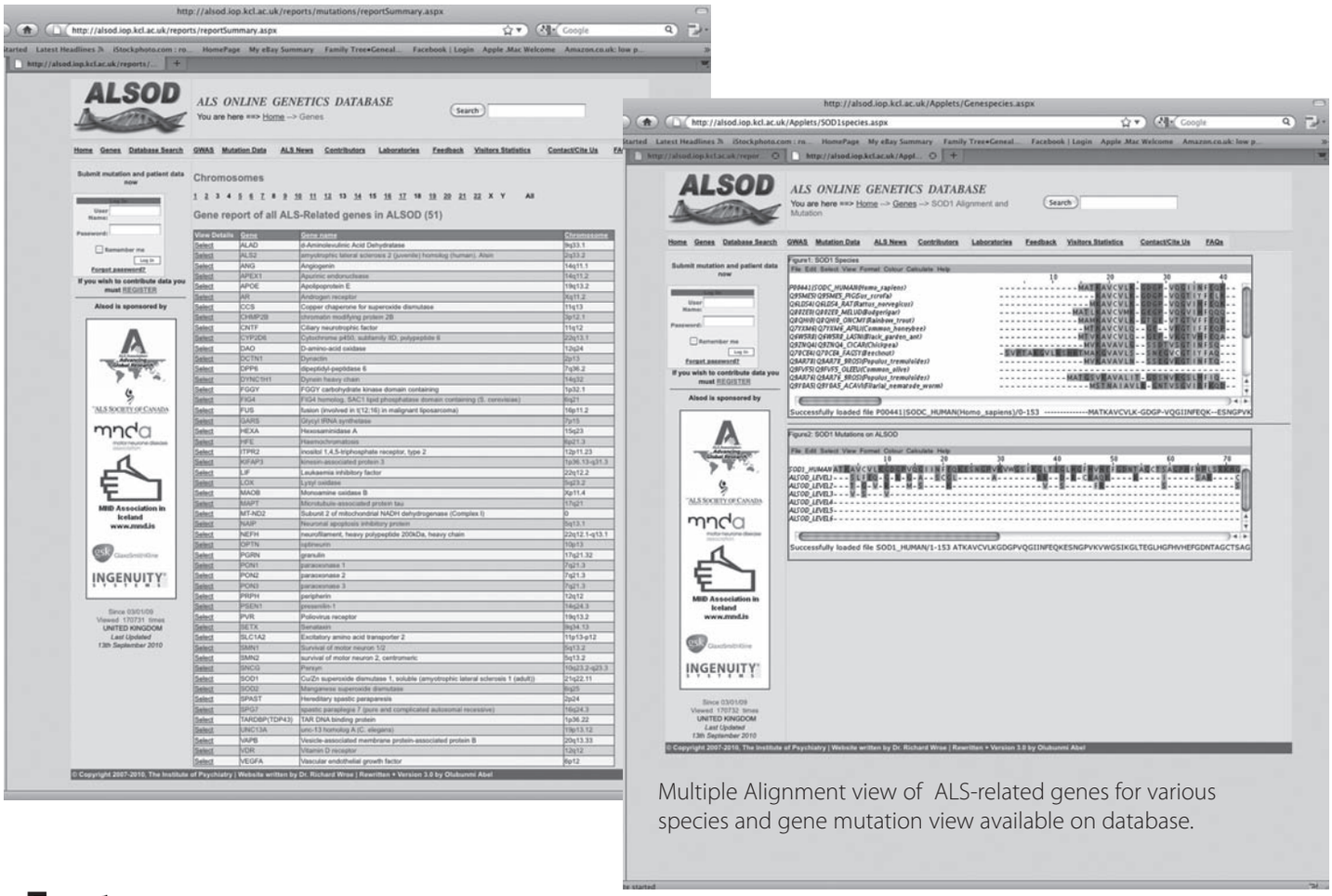
The ALS Online Genetics Database

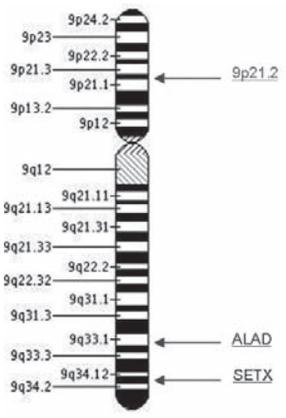
http://alsod.iop.kcl.ac.uk



Features:

- **Statistically compare your genome-wide association results with published genetic studies**
- **Find key literature for each genetic finding (familial and sporadic)**
- **Get other bioinformatics links to each gene (not just SOD1) and their key publications**
- **Study the geographical distribution of published mutations**

A chromosomal overview of all ALS-related genes



ALS Association
Fighting Lou Gehrig's Disease

mnda
motor neurone disease
association

ALS ASSOCIATION

MND Association in Iceland
www.mnd.is

gsk GlaxoSmithKline

INGENUITY SYSTEMS

International Alliance of ALS/MND Associations

Do you know of an ALS/MND Association that could be a member of the International Alliance?

Do you know a key person who might be interested in receiving information about starting an organisation to support people living with ALS/MND?

The International Alliance of ALS/MND Associations has a goal to increase the number of organisations that are working with and supporting people living with ALS/MND around the world.

We have exhausted Google and other search options, and now we call on you, researchers and health professionals, to assist us. We need you to identify and provide contact details for new and emerging ALS/MND organisations, or individuals who may play a key role in establishing such organisations.

Our aim is to offer them support and the opportunity to be a part of a global organisation, and to draw on the support and information that a global organisation can provide to assist them to fight ALS/MND.

If you know of an organisation, or of a key person who may have interest in starting an organisation, please advise the Alliance of their details to alliance@alsmndalliance.org.

Alternatively, ask them to visit www.alsmndalliance.org and see what is available to member organisations through the Alliance.

We need your help - think hard!

APPLICATION FOR MEMBERSHIP FOR THE WFN/ALS WORKING GROUP 2010

First name _____ Surname_____

Department_____

Institution/Company_____

Street/P.O. Box _____

Postal code_____ City _____

State/Country _____

Phone: _____ Fax _____

E-mail (required):_____
(Please complete all the details above.)

Please tick the box with the description that is applicable to you:

- ❑ Clinician (MD)
- ❑ Clinician researcher (MD, PhD)
- ❑ Clinician (Nursing/Allied professional)
- ❑ Other
- ❑ Laboratory scientist

The **membership fee for 2010 is USD 235**, and you will not be fully accepted as a member until that amount has been paid to Informa UK Ltd.

Membership includes the journal *Amyotrophic Lateral Sclerosis* both in printed version and as online journal. Postage is included. Online acccess can be activated via e-mail (see address below) as soon as the membership fee has been paid. Payment details is to be found below.

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Informa Healthcare Customer Services
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Tel: +44 (0)20 7017 5540
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