GEN-01: An Interval Look at Early Markers of Disease in Familial ALS

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Background:
The validation of sensitive biofluid markers of inciting disease pathology and clinical outcomes is crucial to detecting early disease, and to understanding the onset of familial ALS and preparing for successful prevention trials in asymptomatic gene carriers.

Objective:
To identify the earliest biological markers of disease in pre-symptomatic ALS gene carriers

Methods:
Participants are enrolled in the Dominant Inherited ALS (DIALS) Network at Massachusetts General Hospital and Washington University. Genetic testing of over 30 ALS causative genes and potential genetic modifiers is performed, and participants return every 6 months for clinical outcomes and biofluid collection (plasma, serum, whole blood, CSF, PBMCs, urine, skin biopsy). Clinical data is collected using NeuroBANK.

Interval biofluid marker analysis includes longitudinal assessment of neurofilament light chain, chitinases, and dipeptide repeat proteins in collaboration with the Petrucelli and Gendron Labs at the Mayo Clinic in Jacksonville FL and the Bowser lab at the Barrow Neurological Institute. In symptom converters, these candidate biomarkers are directly compared to clinical outcomes of strength (ATLIS), bulbar function (quantitative speech analysis and IOPI), and disease progression (change in ALSFRS-R total score over time). Multiple measures of strength (ATLIS, HHD) and cognition (NIH examiner, ALS-CBS) will be evaluated in all participants over time.

Results to date:
Interval analysis will be shown for 86 participants enrolled in the DIALS Network. Forty-one of 86 participants are positive for an ALS causative mutation (33 C9orf72, 5 SOD1, 1 TARDBP, 1 VAPB, and 1 FIG4). Peri-conversion data will be shown for the 4 C9orf72 carriers (3 ALS, 1 FTD) who have developed symptoms. Longitudinal strength, bulbar, and cognitive outcome data as well as direct biomarker comparison will be shown. DIALS enrollment and follow-up visits are ongoing.

Discussion:
The DIALS Network dataset is composed of a growing number of pre-symptomatic ALS gene carriers, now including several symptom converters. Increased sample size and longitudinal evaluation will continue to inform key elements of the conversion period critical to the design of prevention trials in familial ALS.

Acknowledgements:
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GEN-02: Blood Transcriptomics of ALS Patients in the IMODALS Clinical Trial

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Background:
A spreading inflammatory condition within the CNS, involving both resident and peripheral immune cells, is characteristic of amyotrophic lateral sclerosis (ALS). Regulatory T cells (Treg) are immune regulators which suppress the establishment of excessive inflammatory conditions and autoimmune disorders. These cells are dramatically and progressively reduced in ALS patients and this correlates with the rate of disease progression. Moreover, evidence shows their functional impairment in ALS individuals. Low-dose interleukin-2 (ld-IL-2) has been proposed as a therapeutic strategy to promote Treg expansion and a physiological restoration of the immune balance. This cytokine is crucial for Treg differentiation, activation and functions and thus it is hypothesized to boost Treg-mediated immune-suppression and dampen neuroinflammation in these patients.

Aim:
To evaluate the effect of ld-IL-2 on the blood transcriptome of ALS patients included in the IMODALS clinical trial (NCT02059759).

Methods:
Thirty-six ALS patients were recruited and randomly assigned to three treatment arms: placebo, 1MIU or 2MIU IL-2. They underwent infusions once daily for 5 days every 28 days for a total of 3 months. Blood samples were collected at different time points throughout the trial. White blood cells were isolated and RNA extracted. This was then used to generate microarray gene expression data and for validation through nanoString and qRT-PCR.

Findings:
Blood transcriptome of these patients was analysed at day 8 (after the first treatment cycle), 64 (after the third and last cycle) and 85 (during follow-up) and changes over the treatment course and under different administration regimens were evaluated. Gene expression analysis together with pathway analysis revealed a prompt immune-regulatory effect of ld-IL-2 after the first treatment cycle with evidence of downregulation of pro-inflammatory processes. This early response seemed to diminish during the course of the trial but a robust activation of Treg was only visible after the last treatment cycle. In fact, a time-dependent and dose-dependent induction of Treg markers was registered at day 64 suggesting a cumulative effect of ld-IL-2 on these cells. However, inter-individual dissimilarities were identified and patients were classified into low, mild and high responders. Significant transcriptional differences were reported amongst these groups in their baseline expression of an immunological transcript cluster. In particular, a more inflammatory-prone phenotype was documented amongst low responders at recruitment. Finally, a predictive biomarker analysis was then carried out to identify small sets of transcripts able to forecast an ALS individual's reaction to ld-IL-2. We discovered that the expression of two genes was able to predict Treg count at the end of the trial, given their gene expression at time of recruitment.

GEN-03: Dissecting the sex-dependent genetic architecture of amyotrophic lateral sclerosis

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Sex affects the prevalance[1], site of onset[1], and even heritability[2] of ALS, with evidence that both male[3] and female[4,5] sex hormones modify disease risk. However, to date the extent to which sex affects the genetic architecture of ALS has not been addressed. We calculated the genetic overlap between sexes in ALS pedigrees using published sex-stratified heritability estimates[2], showing an imperfect genetic correlation between males and females of just 62.8% (48%-73.2%). To answer whether this can be explained by common variants, we reanalysed 36,052 samples from a published ALS GWAS[6], accounting for gene-by-sex interactions. Firstly, we ran GREML analysis of ALS heritability fitting sex as an interaction term and observed significant evidence of a gene-by-sex interaction (likelihood-ratio test: p=0.0087) accounting for 1/3rd of the total SNP heritability. Next we ran GWAS using linear mixed model on stratified male-specific (N=18,732) and female-specific (N=17,322) subsets of the data to further dissect sex effects. We estimated heritability using LD-score regression, which showed female-specific heritability (h2=0.0434; SE=0.0144) was significantly higher (p=0.025) than male-specific heritability (h2=0.0023;SE=0.0288). To detect loci differentially associated with risk in males and females, we ran a sex-specific association scan identifying variants significant at a 5% FDR in one sex and not passing nominal significance in the other (p>0.05). Our scan showed that several known (MOBP, C9orf72, SARM1, UNC13A) and novel (PIP5K1B, ATP8A2, PCDH9, RNASE9, OTUD7A, ITPRIPL2, UNK, FBF1) loci harboured SNPs associated with ALS in only one sex. Of these loci, PIP5K1B also showed a significantly different effect size across sexes. Sex dependent loci were significantly enriched in genes highly expressed in the brain, consistent with the known aetiology of ALS. Finally, per-chromosome heritability estimates (GREML) showed a linear relationship with chromosome length in females (r2=0.27;p=0.0074), but not males (r2=0.009,p=0.286) suggesting ALS is more polygenic in females. This observation was supported by analysis using Heritability Estimation from Summary Statistics (HESS), which showed a greater proportion of the genome contributed to ALS heritability in females. Our findings suggest the genetic component of ALS differs between sexes.

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GEN-05: Fine tuning a molecular diagnosis algorithm for patients with ALS and FTD

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Background:
Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) form a clinical, pathological and genetic continuum. The changing scene in molecular diagnosis thanks to NGS development has caused an enormous increase in genetics knowledge in both pathologies. The aim of this study is to evaluate the best molecular diagnostic algorithm enabling an optimal genetic analysis to be performed on ALS and FTD patients worldwide.

Methods:
The number of index ALS or FTD cases in “i+12” biobank is 1336 taking into account familial, sporadic, and comorbidity ALS-FTD cases. This number includes 1156 ALS patients and 180 FTD patients. In turn, a series of 167 patients that form the ALS-FTD group can be excised. We use different NGS approaches. Firstly, we designed a Custom Gene Panel with TruSeq Custom Amplicon v2 (TSCA Illumina®) technology, with an average coverage of 300X and using MiSeq sequencing platform. We included 48 ALS-FTD related genes (indicated in bold in Figure 2).
Secondly, we performed Whole Exome Sequencing (WES) by using the gold standard SureSelectXT Human All Exon V6 (Agilent) technology, with a minimum coverage of 100X and using the NovaSeq6000 (Illumina®) sequencing platform. We analyzed a total of 241 genes increasing the relevance of other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, and other neuromuscular diseases such as Hereditary Spastic Paraplegia (HSP), Charcot-Marie-Tooth (CMT) or Spinal Muscular Atrophy (SMA) too.

Results:
Common related ALS and FTD genes barely contribute small percentages in sporadic diseases, the most notable being C9orf72 for both sALS and sFTD, SOD1 in sALS and GRN in sFTD. These same genes do have great relevance in family groups and in the ALS-FTD group, highlighting in turn the great contribution of C9orf72 (19,0%) and SOD1 (20,3%) in fALS, TARDBP in fALS and fFTD and MAPT in fFTD.

Discussion:
C9orf72 has been systematically related to the 5 differentiated groups in this work, SOD1 with fALS and sALS, and MAPT in fFTD and sFTD. As in these studies, in the design of this algorithm SOD1 is established as a priority in fALS and sALS.
The fact that other recent studies conducted in the Spanish population have related the presence of TBK1 mutations in more frequent percentage than expected in patients with ALS-FTD ratifies its situation within the algorithm. FUS mutations are found in a prominent number of patients with sporadic juvenile ALS.
Surprisingly, TARDBP appeared to a greater extent than expected in cases with familial ALS, so we give it a greater relevance than previously described. WES is performed to include genes that contribute a smaller proportion of patients in each group.

GEN-06: FUS gene is dual-coding with both proteins united in FUS-mediated toxicity

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Abstract:
Ten of thousands of ORFs are hidden within genomes (1). They have eluded annotations because they are either small or within unsuspected locations. Thus, novel functional coding sequences (altORFs) are camouflaged within annotated ones (CDS) in a different reading frame (2). With the mechanism of the wild-type or ALS-linked mutated FUS toxicity still left unclear (3), we hypothesized an overlooked protein within the FUS gene may be at play.

Methods:
We used the proteogenomic resource, OpenProt (1), to query the unbiased coding potential of the FUS gene. We then combined proteomics, ribosome profiling, genomics, bioinformatics and cellular assays to functionally characterize a novel protein. We further validated our findings in a fruit fly model of ALS.

Results:
We discovered an altORF nested in the FUS CDS encoding a conserved 170 amino acid protein, altFUS. AltFUS is endogenously expressed in human tissues, notably in the motor cortex and motor neurons. Overexpression of wild-type FUS and/or amyotrophic lateral sclerosis-linked FUS mutants is known to trigger toxic mechanisms in different models. These include an inhibition of autophagy, loss of mitochondrial potential, and accumulation of cytoplasmic aggregates (4). We show here that altFUS, not FUS, is responsible for the inhibition of autophagy. AltFUS is also pivotal in the mechanisms leading to the mitochondrial potential loss and accumulation of cytoplasmic aggregates. Suppression of altFUS expression in a Drosophila model of FUS-related toxicity protects against neurodegeneration. Some mutations found in ALS patients are overlooked because of their synonymous effect on the FUS protein. Yet we showed they exert a deleterious effect via their missense consequence on the overlapping altFUS protein. These findings demonstrate that FUS is a bicistronic gene and suggest that both proteins, FUS and altFUS, cooperate in toxic mechanisms.

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GEN-07: Genetic and epigenetic disease modifiers in a C9orf72 family presenting with ALS, FTD or PD phenotypes

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Background:
The presence of the GGGGCC hexanucleotide repeat expansion (HRE) in C9orf72 gene is associated to a wide spectrum of phenotypes in the clinical continuum from ALS to FTD, but also including parkinsonisms with a high inter- and intra-familial variability. An Italian family came to our attention because the father was diagnosed with Parkinson disease associated with delusions of paranoid type at the age of 67, while the two daughters were affected, respectively, by FTD at 45 years of age and by ALS at 46 years. Genetic analysis identified C9orf72 HRE in all the affected members, while the mother was wild-type and showed no neurological signs.

Objectives:
Given the observed intra-familial variability in association to C9orf72 gene and the evidence of an earlier disease onset in the two siblings compared to the father, we investigated whether this clinical anticipation could be explained by additional genetic and epigenetic modifiers.

Methods:
Southern blot analysis was used to determine HRE size, while targeted next generation sequencing to analyze 62 different genes associated to ALS/FTD/AD and PD. Analysis of DNA methylation was performed by bisulfite sequencing of the 26 CpG sites within the CpG island of C9orf72 promoter.

Results:
Analysis of C9orf72 HRE length in peripheral blood by Southern blot revealed that all affected members carried the same repeat expansion of about 1500 units. The study of epigenetic modifications showed that the 26 CpG sites within C9orf72 promoter were unmethylated in all family members. Finally, our targeted sequencing of 62 genes associated with different neurodegenerative diseases revealed that the father and the daughter with FTD carried also a heterozygous variant in ATP13A2 gene (p.Ile946Phe), previously described in a patient with early-onset PD. In addition, the father also showed a heterozygous EIF4G1 variant (p.Ala13Pro), that might increase his susceptibility to develop PD.

Discussion:
Our findings confirm that C9orf72 HRE is associated to a wide intra-familial phenotypic variability and show that neither HRE length nor promoter methylation act as disease modifiers within this family, at least in blood, not excluding HRE mosaicism and a different methylation pattern in the brain. However, our data seem to support an oligogenic inheritance model where, in addition to C9orf72 HRE, the presence of other rare variants in genes associated to neurodegenerative disorders may influence the clinical manifestations within the same family.

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GEN-08: Mutational analysis of known ALS genes in an Italian population-based cohort

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Objective:
To assess the burden of rare genetic variants and to estimate the contribution of known ALS genes in an Italian population-based cohort we performed whole-genome sequencing in 959 ALS patients and 677 matched healthy controls.

Methods:
We performed genome sequencing in a population-based cohort (Piemonte and Valle d'Aosta Registry for ALS, PARALS). A panel of 40 ALS genes was analyzed to identify potential disease-causing genetic variants and to evaluate the gene-wide burden of rare variants among our population.

Results:
A total of 959 ALS patients were compared with 677 healthy controls from the same geographical area. Gene-wide association tests demonstrated a strong association with SOD1, whose rare variants are the second most common cause of disease after C9orf72 expansion. A lower signal was observed for TARDBP, proving that its effect on our cohort is driven by a few known causal variants. We detected rare variants in other known ALS genes that did not surpass statistical significance in gene-wise tests, thus highlighting that their contribution to disease risk in our cohort is limited.

Conclusions:
We identified potential disease-causing variants in 11.9% of our patients. We identified the genes most frequently involved in our cohort and confirmed the contribution of rare variants in disease risk. Our results provide further insight into the pathological mechanism of the disease and demonstrate the importance of genome-wide sequencing as a diagnostic utility.

GEN-09: RNA-seq profiling of nuclear SOD1 concentration-dependent pathways in Peripheral Blood Mononuclear Cells of Sporadic Amyotrophic Lateral Sclerosis patients

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Background:
Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disease that can occur sporadically, without any family history (sALS; 90-95% of patients), or with a familial history (fALS; 5-10%). Among the genes reported in ALS pedigrees, there is strong evidence supporting a pathogenic role for SOD1. The involvement of wild-type SOD1 in sporadic cases has been investigated and it was demonstrated that depending on SOD1 localization, sALS patients can be divided into two subgroups: those where the protein aggregates in cytoplasm and those where it relocates in nuclear fraction (1). The protein re-localization as aggregates in insoluble fraction generates oxidative stress leading to DNA damage in contrast with the protective role that SOD1 acquires in the nucleus, preventing DNA damage (2). RNA metabolism is relevant in ALS disease etiology (3). Issues in RNA processing have been associated to ALS (4) and in fact changes of gene expression in patients have been demonstrated (5;6).

Objectives:
To investigate the role that SOD1 exerts in the nucleus of sALS patients by studying pathways activated when the protein concentrates in this compartment.
Methods:
We investigated pathways activated by nuclear SOD1 (nSOD1) in Peripheral Blood Mononuclear Cells (PBMCs) of sALS patients by dividing them depending on the "high" or "low" concentration of nSOD1. PBMCs from sporadic ALS patients (n=18) and healthy controls (n=12) were collected to perform RNA-seq experiments and differential expression analysis.
Results:
We obtained two gene expression patterns for high and low nSOD patients. Differentially expressed genes in patients with high nSOD1 form a cluster closer to controls compared to low nSOD1 group.
Discussion:
Pathways activated in high nSOD1 are related to the up-regulation of HSP70 ensuring the correct protein folding. HSP70s up-regulation has been associated to mutant SOD1 aggregation suppression and their involvement in molecular network of ALS also includes TDP-43 clearance. In low nSOD1 group the up-regulation of KDM4C and S100B may be responsible for loss of DNA damage sensing and increased neuroinflammation. Our findings highlight the importance of subcellular localization of soluble SOD1 in ALS patients. We observed different behavior of RNA regulation in the two groups of patients, leading to pathways conferring "protection" where nSOD1 was high, and "perturbation" in crucial biological systems where nSOD1 was low.
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GEN-10: Simultaneous ALS and SCA2 associated with an intermediate-length ATXN2 CAG-repeat expansion

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Background:
Spinocerebellar ataxia type 2 (SCA2) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative diseases with a common molecular basis: both are associated with CAG-repeat expansion of ATXN2 and with the development of TDP-43-positive neuronal cytoplasmic inclusions. Interestingly, the age of onset of SCA2 is dependent upon expansion length but the age of onset of ATXN2-ALS is independent of expansion length. To date, the two disorders are viewed as clinically distinct with ALS resulting from 30-33 CAG-repeats and SCA2 from >34 CAG-repeats.

Case Description:
We describe a 67-year old female who presented with simultaneous symptoms of ALS and SCA2. Genetic profiling revealed a 32 CAG-repeat expansion of ATXN2 which is close to the borderline between expansion sizes associated with ATXN2-ALS and SCA2.

Conclusion:
Our case demonstrates that the clinical dichotomy between SCA2 and ATXN2-ALS is false. We suggest that CAG-repeat expansion length determines the timing of SCA2 clinical symptoms relative to onset of ALS. In our interpretation patients with a shorter expansion develop ALS before any symptoms of SCA2; patients with a longer expansion develop SCA2 at an early age but if they survive until the typical age of onset for ALS they may then develop superimposed motor neuron degeneration. Our patient with a borderline-length expansion developed both disorders simultaneously. Review of the literature and our local cohort provides evidence for occurrence of ALS in late stage SCA2, which may be under-recognised by clinicians who think of the two diseases as separate processes.

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GEN-11: Telomere length analysis in 6,500 ALS whole genome sequences from Project MinE

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Background:
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative syndrome characterized by the degeneration of upper and lower motor neurons, which lead to muscle weakness and paralysis eventually. ALS is an umbrella term used to describe ALS phenotype including progressive muscular atrophy, primary lateral sclerosis, and other clinical manifestations such as bulbar palsy. ALS shares pathobiological features with frontotemporal dementia. There is a strong genetic contribution to ALS risk. In 10% of cases or more, a family history of ALS or frontotemporal dementia is obtained, and the Mendelian genes responsible for ALS in such families have now been identified in about 70% of cases. Only about 11% is explained by common gene variants, suggesting that other forms of genetic variation are important. Telomeres maintain DNA integrity during cellular replication and shorten naturally with age. Gender and age are risk factors for ALS and also associated with telomere length. We therefore investigated telomere length in ALS.

Methods:
Samples were from Project MinE, an international whole genome sequencing ALS consortium. Ancestry and relatedness were evaluated by principal components analysis and relationship matrices of DNA microarray data. Whole genome sequence data were from Illumina HiSeq platforms and aligned using the Isaac pipeline. We estimated telomere length by applying a bioinformatics analysis to whole genome sequence data of leukocyte-derived DNA from people with ALS and age and gender-matched matched controls. We tested the association of telomere length with ALS and ALS survival.

Findings:
There were 6,580 whole genome sequences, reducing to 6,195 samples (4,315 from people with ALS and 1,880 controls) after quality control. Accounting for age and sex, there was a 20% (95% CI 14%, 25%) increase of telomere length in people with ALS compared to controls ($p = 1.1 \times 10^{12}$). Those with shorter telomeres had a 10% increase in median survival ($p = 5.0 \times 10^{7}$). Analysis of ALS subgroups shows that the mean telomere length in progressive bulbar palsy was on average 39% (95% CI 9%, 70%) more than the mean sporadic ALS telomere length (p=0.009). Furthermore, we compared telomere length between sporadic ALS and familial ALS and no deference was observed (p=0.64).

Conclusions:
It is likely that longer telomeres increase risk for ALS. Furthermore, we have shown that telomere length varies greatly between ALS subgroups and affect survival. Therefore, telomere length is a potential biomarker and should be considered as a target for therapy in ALS.

GEN-12: Clinical Characteristics of SOD1 and c9ORF72 positive ALS: Findings from the Canadian Neuromuscular Disease Registry

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Background:
The Canadian Neuromuscular Disease Registry (CNDR) is a nationwide neuromuscular disease registry. The CNDR collects data in affiliated clinics across Canada and currently has over 4100 total and 1500 ALS patients registered. Therapies for genetic-based ALS are under development targeting two of the most common mutations in ALS, c9ORF72 and SOD1. The objective of this study is to characterize SOD1+ and c9ORF72+ ALS patients in Canada.

Method:
Data from consenting ALS patients were recorded at 13 affiliated ALS clinics across Canada. Patients with a known SOD1 or c9ORF72 mutation and gender- and age-matched control individuals without a known genetic mutation or family history of ALS were included in the analyses. Progression rates were calculated in the first-year post-diagnosis by change in ALSFRS-R/time. Patients without multiple visits within the first-year post-diagnosis were excluded from progression analyses. Similarly, patients whose ALSFRS-R scores coincided with clinical trial participation were excluded from progression analyses.

Results:
Baseline data from 27 SOD1 patients, 38 c9ORF72 patients, and age- and gender-matched controls without a family history or known genetic mutation are presented. Median age at diagnosis was 58.3, 59.6, and 59.5 years for SOD1, c9ORF72, and controls respectively. SOD1 patients were 54% male, c9ORF72 65%, and controls 63%. Mean baseline ALSFRS-R at diagnosis (within 4 months post-diagnosis) were 39 (range: 23-48) for SOD1, 39 (range: 26-48) for c9ORF72, and 40 (24-47) for controls. Of the 25 SOD1 patients with mutation recorded, 48% had the Ile113Thr mutation. Progression analyses was performed on the subset of 17 SOD1 cases, 33 c9ORF72 cases, and 50 controls with complete data, and without clinical trial participation. ALSFRS-R progression rates were 1.0 points/month (range: 0-2.8) for SOD1, 1.2 points/month (0-3.4) for c9ORF72, and 0.9 points/month (0-2.4) for controls. Further longitudinal analyses incorporating potential confounders will be presented.

Discussion:
The CNDR is a nationwide platform for quantifying the burden of illness and describing clinical care and outcomes in Canada. Preliminary results suggest age at onset and disease progression during the first-year post-diagnosis are comparable among SOD1, C9orf72, and sporadic ALS.

Acknowledgements:
Funding support was provided by Biogen. The CNDR would like to thank all patients who participate to make this research possible.

GEN-13: Genome-wide Identification of the Genetic Basis of Amyotrophic Lateral Sclerosis

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Background:
Genetic association studies suffer from poor statistical power which limits discovery. To address this we have integrated functional genomics with disease genetics in a hierarchical Bayesian model called 'RefMap'. Amyotrophic lateral sclerosis (ALS) is an archetypal complex disease centred on progressive death of motor neurons.

Methods and Results:
Functional and epigenetic profiling of iPSC-derived motor neurons enabled RefMap to fine-map causal genes and pathways in ALS. We identified 690 candidate ALS genes; our list is enriched with known ALS genes ($p=0.015$, Fisher exact test) and biological functions linked to the neuromuscular junction ($p<0.0001$, Fisher exact test). Moreover, identified genes are upregulated in undiseased motor neurons but progressively downregulated in patient tissue and ALS disease models ($p<0.0001$, t-test). The most significant new ALS gene is KANK1, which is enriched with coding and non-coding, common and rare, ALS-associated genetic variation ($p<0.05$, SKAT). CRISPR/Cas9 perturbation proximate to patient mutations in a human neuronal cell line reduces KANK1 expression ($p<0.05$, t-test) supporting a proposed loss of function mechanism.

Discussion:
Refmap can be applied broadly to increase power in genetic association studies of complex human traits. In the context of ALS we have identified an extended set of candidate genes including KANK1 which we propose as a new ALS risk gene.

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GEN-14: Heterogeneous motor neuron phenotypes stemming from KIF5A mutations

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The Kinesin Family member 5A (KIF5A) gene encodes for a heavy chain of Kinesin-1 that acts as a microtubule motor in neuronal intracellular transport. The phenotypic spectrum of its mutations is quite varied, and ranges from the hereditary spastic paraplegia (HSP) type 10 to the Charcot-Marie Tooth disease type 2 (CMT2), even comprising a neonatal intractable myoclonus.
Recent studies have disclosed a link between mutations of KIF5A and the development of amyotrophic lateral sclerosis (ALS), as well as of other slow-progressing motor syndromes. While the KIF5A mutations described in HSP or CMT2 patients mainly affect the N-terminal domain of the protein, the ALS-associated mutations seem to affect preferentially its C-terminal domain, with increasingly overlapping phenotypes when mutations affect the stalk domain of the protein. Here we describe two cases of KIF5A mutations with two different phenotypes.
The first case involves a 64 -years old Italian man suffering from a four-year history of gait disorder with stiffness in the lower limbs. His personal history revealed bilateral hearing loss and bilateral cataracts. Neurological examination showed a spastic paraparetic gait, lower limb hyperreflexia with Babinski sign and spastic hypertonia. Brain and spine MRI and neurophysiological tests were normal, except for a prolonged central motor conduction time at motor evoked potentials. A next-generation sequencing panel for HSP revealed a heterozygous c.1630C>T mutation (p.Arg544Ter) in the stalk domain of the protein, introducing a premature stop codon in exon 15 and leading to the production of a truncated protein.
The second case involves a 71 years-old man who came to our attention for a five years history of slowly progressive upper limbs pseudo myopathic proximal hyposthenia with marked upper limb-girdle amyotrophy. While still walking, no signs of upper motor neuron involvement have been detected so far. Brain and spine MRI did not show remarkable changes, while an electromyography showed chronic neurogenic alterations with active denervation in right deltoid muscle, right common digital extensor and first muscle bilaterally, with some fasciculation potentials also highlighted in lower limbs. All the serum tests were negative. An NGS panel for axonal peripheral neuropathies showed a heterozygous c.2301 C>T mutation in KIF5A for which a Sanger test confirmation has already been scheduled.
These cases confirm that overlapping phenotypes with a clinically heterogeneous spectrum of diseases may emerge from KIF5A gene mutations, since it is a pan-neuronal expressed gene regulating axonal functioning. Our findings seem to support the hypothesis that stalk domain mutations may be more likely to interfere with the correct structure of the protein and affect both motor and binding functions, explaining the overlapping and heterogeneous phenotypes described so far.

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GEN-15: Interaction between ALS Polygenic Risk Score and rare variants in ALS genes

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Background:
Amyotrophic lateral sclerosis (ALS) has substantial heritability, with rare variants contributing to familial inheritance of the disease. However, only a small portion of cases are explained by known genetic factors. Genome-wide association studies (GWAS) have implicated several genomic loci in both the risk and severity of ALS. Genetic risk for ALS could be related to a combination of common small effect-size alleles and penetrant rare variants. In the current study, we aimed to test whether the difference in additive common variant risk for ALS was affected by the presence of rare variants in known ALS genes.

Methods:
Samples from 330 ALS cases and 349 unaffected controls collected from clinics across Québec, Canada were included in the study. We used ALS GWAS summary statistics (van Rheenen, et al. 2016) as base data to estimate a polygenic risk score (PRS) for ALS status using the PRSice-2 software. SNP-chip genotyping data was generated for all samples using the Illumina Infinium Global Screening Array v2.0. PRS for other neurodegenerative diseases (Alzheimer's disease and Parkinson's disease) were also calculated to assess ALS PRS specificity. A targeted-sequencing panel or whole exome sequencing was used to detect rare variants in 26 previously associated ALS genes. Variants with minor allele frequency less than 0.1% and predicted to alter protein sequence were included. Samples were grouped into cases or controls and subdivided into rare variant carriers or non-carriers. ROC curves were calculated to assess the sensitivity and specificity of the ALS PRS with respect to carrier status.

Results:
4,309 SNPs were included in the final PRS calculation from a threshold of p = 0.00295 from the base data, explaining 1.16% of variation between cases and controls. The upper quartile of PRS showed an odds ratio of 2.05 for ALS risk. When stratifying by rare variant carrier status, no statistical difference was observed in PRS between variant-carrying ALS cases and carrier controls; however, a significant difference was observed between non-carrier cases and non-carrier controls (p = 0.0087). PRS calculated on the same sample set for other neurodegenerative diseases did not significantly differentiate between ALS cases and controls, nor were they affected by rare variant carrier status for ALS genes.

Discussion:
The ALS PRS was informative only between cases and controls not carrying a rare variant, suggesting that rare variants in ALS genes have a substantial effect on disease risk. Rather than modifying the penetrance of rare risk variants, ALS PRS differentiates cases and controls only in the absence of these variants. While much of the genetic liability for ALS remains to be explained, our results suggest that common variant risk should be considered for cases that are not genetically explained.

GEN-16: Large-scale epigenome-wide association study of ALS: a collaborative effort within Project MinE

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Epigenetic mechanisms, including DNA methylation and histone modifications, may explain how genes and the environment interact and contribute to the onset and progression of ALS. DNA methylation is the best characterized epigenetic modification that stably influences gene expression and is influenced by environmental exposures, genetic variation and stochastic processes. To elucidate the role of DNA methylation in ALS pathogenesis, we have generated methylomic data for 10,598 Project MinE blood samples collected across 14 countries (2:1 case/control ratio) using Illumina 450k and EPIC arrays. In addition, genetic and environmental/lifestyle data were collected for the majority of these samples and as such provides a unique integrated dataset to study the ALS methylome.
Following thorough quality control, we performed epigenome-wide meta-analyses across four cohorts to identify differentially methylated sites, while adjusting for both known and unknown confounders.
At an experiment-wide significance threshold (P < 9 x 10^-8), we identified 45 sites across the genome that were associated with ALS, which were annotated to 40 genes based on eQTM and nearest-gene mapping. We performed extensive sensitivity analyses to confirm that the results were not affected by technical factors such as batch effects and cross-hybridization or biological factors such as local genetic variation and medication use. Gene set analysis revealed that the significant sites were enriched among genes involved in metabolic pathways, and in line with this, we found that these sites significantly overlap with sites reported in previously published EWASs on various metabolism-related traits. In addition, we found a significant overlap with sites reported in allergy and inflammation-related EWASs. To gain further insight into potential intermediate phenotypes driving the EWAS results, we applied published poly-methylation scores (PMS) and found that the PMSs for smoking, alcohol, BMI, HDL cholesterol and white blood cell proportions were independently associated with ALS. These results are a clear validation of results from previous epidemiological studies in ALS. Currently, we are investigating the association between DNA methylation differences on overall survival by performing EWASs on predicted composite survival outcome.
To conclude, in one of the largest case-control EWASs to date, we identified novel associations between DNA methylation and ALS and implicate potential underlying pathways and intermediate phenotypes.

GEN-17: Linking brain cell-subtype specific transcriptomic and epigenomic mosaicism in an ALS/FTLD family with generational C9orf72-repeat instability

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Hexanucleotide repeat expansions of GGGGCC in C9ORF72 (C9) are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Approximately 50% of patients with ALS also exhibit signs of cognitive dysfunction caused by frontal and temporal lobar degeneration (FTLD), with ~15% of cases fulfilling the clinical diagnostic criteria of FTD. The neurodegenerative cascade in C9-ALS/FTLD is non-cell autonomous, whereby astrocytes, microglia, and oligodendrocytes contribute to disease. Post-mortem analyses of ALS/FTLD has indicated brain regional and cell subtype-specific nuclear abnormalities such as DNA damage accumulation, chromatin complex remodeling, and depletion of TDP-43. We have clinically, genetically, and neuropathologically characterized an ALS/FTLD family (PED25) with significant C9-repeat instability in germline and somatic tissues. One PED25 parent presented with normal C9 alleles, whereas the other carried a 70-repeat C9 allele in blood and mosaic repeat length across different brain regions and peripheral tissues but was unaffected by ALS or FTD. Remarkably, four of the five offspring presented with ALS/FTLD and carried ~1750 repeat expansions in blood and one offspring showed normal C9 repeat size. To better understand the brain cell subtype-specific mechanisms resulting from this C9 repeat mosaicism in PED25, we generated single-nucleus (sn) RNA- and assay for transposase accessible chromatin (ATAC)-sequencing libraries from the orbitofrontal cortex from the parent carrying 70 repeats, two of the affected offspring, and compared these with three non-neurological disease controls. The results from both technologies demonstrated widespread, cell subtype-specific alterations across all recapitulated major subtypes in the offspring when compared with both the parent and controls. An integrated analysis of the snRNA- and snATAC-Seq data also indicated both a convergence and divergence in the cell subtype-specific gene expression and corresponding regions of chromatin showing differential accessibility in the PED25 progeny. Overall, these results provide an orbitofrontal cortex atlas of the transcriptomic and epigenomic markers of cell subtype-specific susceptibility and resilience to C9-ALS/FTLD from a family with mosaic C9orf72 repeat instability.

GEN-18: NEMF mutations that impair ribosome-associated quality control are associated with neuromuscular disease in mice and humans

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A hallmark of neurodegeneration is defective protein quality control. The ribosomal quality control (RQC) pathway is an important component of protein quality control; it recognizes stalled ribosomes, facilitating ubiquitination and proteosomal degradation of nascent polypeptides[1]. Whether neurodegeneration results from defective RQC and whether defective RQC contributes to human disease has remained unknown. Here we show that three independently-generated mouse models with mutations in a component of the RQC complex, NEMF, develop progressive motor neuron degeneration. We also identify NEMF mutations expected to interfere with function in nine patients from seven families presenting juvenile neuromuscular disease. These uncover NEMF's role in translational homeostasis in the nervous system and implicate RQC dysfunction in causing neurodegeneration.

Objectives:
To characterize the neurodegenerative effects of mutations in Nemf in mice and to identify human patients harboring NEMF mutations.

Methods:
Two novel Nemf mutations, R86S and R487G, were identified in separate ENU-mutagenesis screens in mice. A putative null, D106*, was produced via CRISPR-Cas9 mutagenesis. Nemf mutants were assessed for overt and histological motor phenotypes. The genomes or exomes of nine patients exhibiting juvenile neuromuscular disease were screened for possible pathogenic mutations.

Results:
All three Nemf mutations resulted in progressive neurodegeneration. Homozygous null mutants displayed severe paralysis and death prior to two weeks of age. Mice homozygous for R86S had an intermediate phenotype characterized by progressive paralysis and death prior to three weeks of age. Homozygous R487G mice displayed a milder phenotype, with slight hind-limb wasting apparent post-wean. Each mutation resulted in degeneration of motor neuron axons and denervation of neuromuscular junctions. Nine human patients harboring mutations in NEMF presented with a variety of abnormal neuromuscular and neurological phenotypes, including axonal neuropathy, ataxia, hypotonia, speech delay, and intellectual disability. Seven patients harbored biallelic variants, an eighth patient harbored an inherited variant and a de novo variant, and the ninth patient harbored a single de novo missense variant.

Discussion:
Our studies identify Nemf as a novel gene implicated in neurodegeneration and neuromuscular disease in mice and humans. This finding strongly points to RQC as a critical molecular pathway protecting neurons against degeneration. This suggests that the presence of variants in NEMF and other RQC factors should be examined in patients with similar diseases.

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GEN-19: Novel FIG4 variants associated with Chinese sporadic amyotrophic lateral sclerosis patients with slow progression

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Background:

Mutations in the FIG4 gene have been linked to ALS11 in Caucasian populations.

Objectives:

The purpose of this study was to identify FIG4 mutations in a cohort of 15 familial ALS indexes and 275 sporadic ALS patients of Chinese origin.

Methods:

All 23 exons of the FIG4 gene were sequenced by targeted next generation sequencing. An extensive literature review was performed to detect genotype–phenotype associations of FIG4 mutations.

Results:

No FIG4 variants were identified in familial ALS. One novel heterozygous missense variant c.352G>T (p.D118Y), one novel nonsense variant c.2158G>T (p.E720X), and one known heterozygous variant c.2661dupG (p.Q888Afs*34) in the FIG4 gene was each identified in one sporadic ALS patient. The p.E720X variant is interpreted as likely pathogenic, the p.D118Y variant is a variant of uncertain significance, while the p.Q888Afs*34 variant is likely to be benign. Mutation frequency of FIG4 is low in our ALS cohort. The patient carrying p.E720X mutation developed lower limb onset slowly progressive ALS at the age of 62, with a survival duration of 11.5 years. The patient carrying p.D118Y variant had an upper limb onset at age 59 and progressed slowly.

Discussion:

The findings suggest that patients with FIG4 mutations are more likely to have a relatively slow progression and a long survival.

GEN-20: The clinical significance of amyotrophic lateral sclerosis and frontotemporal dementia associated genetic variation: a comprehensive, uniform analysis of three decades of genetics research.

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Background:
ALS has a large genetic component with a heritability of approximately 53% and up to 20% of patients presenting with a positive family history. In the 27 years since the discovery of segregating variants in SOD1, many publications have documented potential ALS-associated genes and variants, with varying degrees of supporting evidence. In more recent years, next-generation sequencing has led to a deluge of reported rare variants in previously linked ALS genes. These genetic screening studies have explained, at most, 70% of familial cases and 15% of sporadic cases; however many of these “explanatory” variants are likely to represent non-pathogenic rare variation. The difficulty of interpreting the clinical significance of rare variants is exacerbated in ALS and FTD due to genetic heterogeneity, late age of onset, incomplete penetrance and a high proportion of sporadic cases. Here we present journALS, a comprehensive analytical platform designed to assess the clinical significance of all previously reported amyotrophic lateral sclerosis (ALS)- and frontotemporal dementia (FTD)-associated genetic variants.

Methods:
We reassessed all published evidence supporting ALS- and FTD-associated genetic variants in the context of modern reference data representing benign genetic variation and guidelines for the classification of medically relevant genetic variants. A PubMed, ClinVar and Human Gene Mutation Database search identified 2,914 primary research articles for screening, of which 1,028 were found to be relevant ALS or FTD genetic studies. This primary corpus contained 3,112 reported variants in 363 genes. Detailed phenotype data including sex, age of onset and family status were gathered, in addition to variant information such as zygosity and de novo status.

479 pedigrees exhibiting segregation were documented. Variants in the 363 genes identified in the literature were extracted from publicly available, ALS-specific genomics datasets, creating a final database of 1.5 million variants. Large-scale genomics resources such as gnomAD provide high-resolution rare variant allele frequencies and gene-specific properties. By leveraging these datasets in conjunction with the American College of Medical Genetics variant classification guidelines, we uniformly assessed all variants for pathogenicity, penetrance, prevalence, and phenotypic and geographic heterogeneity.

Results:
91 pathogenic and likely pathogenic variants were confirmed in 24 genes, with 10% classified as benign or likely benign; and greater than 89% classified as variants of uncertain significance.

Discussion:
As precision treatments targeting specific ALS-causing mutations in specific patients are becoming an increasingly important therapeutic paradigm , distinguishing truly pathogenic ALS and FTD variants from benign genetic variation is now essential.

Our results support a reorientated view of several ALS genes and genetic variants for which the published evidence depends heavily on only a single domain. We provide all supporting evidence and analyses in an interactive user-friendly format for clinicians and researchers.

GEN-21: The genetic burden in an Italian cohort affected by Amyotrophic Lateral Sclerosis: the expertise of the diagnostic lab

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Background:
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder (ND) characterized by heterogeneous clinical manifestations due to both genetic and phenotypic overlapping with other NDs, such as Frontotemporal dementia (FTD), Hereditary spastic paraplegia (HSP), Parkinson's disease (PD) and other motor neuron diseases (Elert-Dobkowska et al., 2019; Abramzon et al., 2020). ALS is now considered a polygenic disease, characterized either by oligogenic inheritance and genetic pleiotropy which may lead to complex phenotypic traits in patients (Hardiman et a., 2017).

Objectives:
In this study, we investigated the genetic burden in the ALS clinical spectra and the weight of the presence of genetic variants in genes associated with FTD, HSP, PD and other motor neuron and ND diseases.

Methods:
Next-Generation Sequencing (NGS) was done on 277 sporadic ALS patients, using four different gene panels (Agilent Technologies) of 97, 104, 173 and 277 genes involved in ALS, FTD, HSP, PD and other motor neuron diseases. The eVai (https://evai.engenome.com) software was used for variant calling and classification. Pathogenic, likely pathogenic and uncertain variants (VUS) were retained and validated by Sanger sequencing. C9orf72 repeat expansion was assessed using the repeat-primed PCR assay, as described by DeJesus Hernandez et al. (DeJesus Hernandez et al., 2011).

Results:
By NGS we found a total of 53 variants considered pathogenic and likely pathogenic in 50 out of 277 patients (18%). Among the 53 variants, 19 (36%) were in ALS-causative and ALS-susceptibility genes, 10 (19%) in genes related to HSP, 13 (24%) were in PD-causative genes while the other 11 (21%) were in genes related to other motor neuron or other ND diseases. Seventeen ALS cases (6,1%) resulted with one pathogenic expanded allele in the C9orf72 gene. All patients C9orf72-expanded were negative for the presence of pathogenic and likely pathogenic NGS variants. Complessively, we found pathogenic genetic bases in 77 patients (28%).

Discussion:
Our study confirmed that the genetic architecture of ALS is extremely complex, highlighting the importance to screen all exomes. Considering a broader genetic spectrum will help in understanding also atypical clinical presentations, ameliorating both the differential diagnosis and the times of diagnosis.

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GEN-22: Variable Number Tandem Repeats associated with ALS

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Background:
Tandem repeat sequences are genomic loci composed of a repeating nucleotide motif of variable length. These repeat sequences may be classed by the length of their repeating motif as short tandem repeats, with motifs of up to 6 nucleotides, or variable number tandem repeats with motifs of >6 nucleotides. To date, more than 40 short tandem repeats are known to cause neurological disease due to repeat expansions, of which 2 have been studied in relation to ALS: a hexanucleotide intronic repeat in C9orf72 which represents the largest genetic cause of the disease, and a trinucleotide repeat in ATXN2 which increases susceptibility to ALS. VNTRs have proven more difficult to study due to their longer repeat length and variable internal structure. However, new sequencing techniques allowed the first VNTR associated with ALS to be identified in 2019, a 69 nucleotide repeat in the last intron of the WDR7(1).

Objectives:
We aim to further characterise the WDR7 VNTR in cohorts of ALS and to identify other VNTRs associated with ALS from these cohorts.

Methods:
We used genomes collected by the ALS consortium of the New York Genome Centre to compare VNTR lengths and composition in ALS patients and controls.

Results:
We validate previous characterisations of VNTR lengths and identify novel VNTRs associated with ALS.

Discussion:
Tandem repeat sequences are a major cause of neurological disease, of which the majority of those characterised have been short tandem repeats. The investigation of VNTRs has the potential to identify novel causes and modifiers of ALS, increasing our understanding of the heritability and pathogenesis of the disease.

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We would like to thank the NYGC ALS consortium.

GEN-23: Combined epigenetic/genetic study identified an amyotrophic lateral sclerosis age of onset modifier

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Age of onset in amyotrophic lateral sclerosis (ALS) patients is highly variable. For example, age of onset in carriers of the C9orf72 G4C2-expansion can vary between 27-74 years. The variable age of onset might be influenced by environmental and genetic factors via the modulation of DNA methylation (DNAm) at CpG-sites, which is one of the key epigenetic modifications modulating gene expression or RNA splicing. Here, we combined an epigenetic and genetic approach to test the hypothesis that single nucleotide polymorphisms (SNPs) at CpG-sites (CpG-SNPs) could affect DNAm levels and modify ALS age of onset. First, our genome-wide DNAm analysis identified 9 CpG-SNPs whose DNAm levels are significantly associated with age of onset in genetically unexplained ALS patients (n=249). Next, a genetic analysis validated an association of rs4970944 with ALS age of onset in the discovery (n=469; P=0.025) and replication (n=4160; P=0.007) cohort. A similar association was observed with its tagging SNPs (rs10888406, rs11204785, rs11807075), implicating the 16 Kb region at the 1q21.3 locus as a modifier of ALS age of onset. Notably, rs4970944 genotypes are also associated with age of onset in 333 C9orf72-carriers (P=0.025). A meta-analysis of all ALS patients (n=4629) suggested that every A-allele of rs4970944 is linked to about one year later onset (P=0.0012), and the median age of onset in AA-carriers was two years later than in GG-carriers (59 vs 57 years). Analysis of Genotype-Tissue Expression dataset revealed that the G-allele of rs4970944 (corresponding to an earlier ALS onset) is significantly associated with elevated expression of CTSS in cerebellum (P=0.00018). CTSS is encoding cathepsin S protein, which plays a key role in antigen presenting. In conclusion, the current study identified a 16 Kb region at the 1q21.3 locus (tagged by rs4970944) as a modifier of ALS age of onset. Our findings support the role of antigen presenting processes in modifying age of onset, and suggest potential drug targets (such as CTSS).

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GEN-24: Evidence for the GPX3/TNIP1 locus and its contribution to the risk of ALS

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Background:
Amyotrophic lateral sclerosis (ALS) is a complex late-onset, neurodegenerative disease with a poor prognosis. We were the first to identify an ALS risk locus on chromosome five using a multi-ethnic GWAS approach in 2017 [1] which was subsequently detected in a larger, partially overlapping, European only ALS GWAS [2]. Current association analysis data alone cannot determine which gene in the locus (two initially implicated GPX3 and TNIP1) is the most plausible contributing to ALS risk. Here, we report on a broad set of follow-up studies conducted in order to provide independent evidence that could support the relevance of one, both or neither in the context of ALS risk.
Objective:
To narrow down on the likely causal gene in an ALS GWAS risk-locus for follow-up studies.
Methods:
We used in-silico (COJO, FUMA, LDSC, PoPS, SMR, TWAS), in-vitro (human motor neurons) and in-vivo (expression in ALS case/control plasma, zebrafish) approaches to narrow down the likely candidate mechanism that alters susceptibility to ALS.
Results:
Both TNIP1 and GPX3 are implicated across a broad range of in-silico analyses, as a reflection that rs10463311 is an eQTL for both genes. The in-vivo expression analyses in ALS cases (preliminary n= 48 and replication n=198) suggests GPX3 protein expression decreases in plasma with worsening disease (ALSFRS-R, adjusted R2 = 0.042, p = 0.0055, replication) with TNIP1 not detected. In-vitro GPX3 and TNIP1 knock-down in differentiated human motor neurons did not identify a phenotype however, functional validation in-vivo indicates a pathogenic role of GPX3 loss-of-function causing motor deficits in zebrafish embryos (not seen in tnip1-MO). Gpx3-MO (1mM) injected animals moved less distance, fewer minutes and with an overall lower speed compared to CTR-MO-injected controls (swim distance, time and speed mean difference and 95% CI: 112 ± 28 mm, 1.29 ± 0.59 secs, 32.0 ± 2.53 mm/s respectively. The behavioural defects were all significantly reduced (rescued) following co-injection with 100pg of MO-insensitive gpx3-mRNA (cst-gpx3).
Discussion/conclusion:
Here we have demonstrated an investigation of a risk locus for ALS to identify a candidate gene for follow-up studies. We use complementary lines of evidence demonstrate support for GPX3 contributing to ALS risk which has implications for understanding mechanisms of disease and targeted therapeutic approaches. We highlight that aspects of this pipeline (particularly in-silico, which is high-throughput and cost-effective) could be utilised for other loci as they are identified to improve the consistency of these studies, which has been hampered due to inherent difficulty of modelling neurodegenerative disease.

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GEN-25: Genetic analysis of GLT8D1 and ARPP21 in Australian ALS cases

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Background:
Recently, genetic sequencing analysis of ALS cases of predominantly European ancestry have linked variants in GLT8D1 (glycosyltransferase 9 domain containing 1) and ARPP21 (cAMP regulated phosphoprotein 21) to familial ALS (FALS) and sporadic ALS (SALS) (1). However, a subsequent report that used a Chinese cohort found no significant association between ALS and GLT8D1 or ARPP21 variants using Fisher's exact testing (2).
Objectives:
We sought to investigate the prevalence of GLT8D1 and ARPP21 variants among Australian patients using whole-genome and whole-exome sequencing data from 618 SALS and 81 FALS cases (from 61 distinct families), all with unknown ALS causative mutations.
Methods:
We developed custom bioinformatics pipelines using UNIX and R to perform gene-based burden analysis using Fisher's Exact testing to determine whether GLT8D1 and/or ARPP21 carried a burden of protein-altering or splicing variants in comparison to two ethnically matched Australian control cohorts and the Genome Aggregation Database (GnomAD). Variants qualified as rare if they had a minor allele frequency (MAF)<0.005 in case/control cohorts, except for GnomAD variants qualifying with MAF<0.000. Moreover, we investigated whether any qualifying variants were overrepresented or underrepresented among cases as compared to controls, and potentially acting as risk or protective alleles for ALS, respectively.
Results:
Results identified an absence of any protein-altering or splicing variants in the GLT8D1 gene among both SALS and FALS cases. Eight such variants were found within ARPP21 (seven in SALS and one in FALS), and three of these were predicted to be pathogenic by at least 4/7 prediction tools. However, all these variants were present in the nNFE GnomAD subset with minor allele frequencies (MAF) of >0.0001 and/or were present in >3 healthy people in all available control databases. None of these variants showed evidence of association with ALS. Gene-based burden analysis of ARPP21 indicated no enrichment of rare protein altering ARPP21 variants in ALS cases when compared to each of three control cohorts . As no qualifying variants were identified in GLT8D1, burden analysis was not performed.
Discussion:
These findings suggest that mutations in GLT8D1 and ARPP21 are not a common cause of ALS in Australia. Further studies with larger sample size and diverse ancestries will be required to validate the potential contribution of GLT8D1 and/or ARPP21 variants to ALS. It is essential to determine the prevalence of novel ALS genes to improve our understanding of disease biology, and also provide guidance for common targets for the development of diagnostics and future treatments.

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GEN-26: Genetic marker development to enrich ALS clinical trials

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Background:
Amyotrophic lateral sclerosis (ALS) is complex neurodegenerative disorder that has extremely diverse clinical presentations and progressions between patients. As a result, one form of treatment is unlikely to be effective in all ALS patients. Understanding the genetics that drives this disease is critical for identifying sub groups of patients that have similar disease pathologies and/or a similar drug response. In particular, characterizing genomic regions known as structural variants may provide insight into different disease mechanisms and explain the variability seen in age-at-disease onset, disease progression and phenotype. Structural variants may inform novel therapeutic targets, or be used as markers for patient stratification to eliminate participant heterogeneity and increase the likelihood of clinical trial success.

Objective:
The aim of this study was to investigate the presence of structural variants within ALS linked or associated genes as genetic markers to determine their association with disease and clinical phenotype.

Results:
To date we have identified a number of SVs in candidate ALS genes that are associated with a range of ALS disease outcomes. (1) Two long alleles (≥19) of a CA repeat within intron 3 of STMN2 is associated with sporadic ALS disease risk ($p = 0.042$), and is heightened with the presence of a 24 CA repeat ($p = 0.0017$). In addition, the presence of one long allele in STMN2 is associated with a 7.5 earlier age-at-disease onset ($p = 0.039$), whilst the L/L & 24 CA repeat genotype decreases survival in a subset of bulbar onset patients ($p = 0.008$). (2) An insertion/deletion in intron 5 of SQSTM1 is associated disease risk in familial ALS ($p = 0.0036$). (3) A poly T variant in SCAF4 is associated with disease risk in sporadic ALS patients ($p = 1.8\text{-}4$) and familial ALS patients ($p = 7.6\text{-}15$), with 26 month reduced survival observed in familial ALS patients carrying the long (18 T) allele ($p = 0.0009$).

Discussion:
These results provide evidence that SVs can be useful genetic markers that can inform disease risk and ALS patient subgroups. Incorporation of these genetic markers into future clinical trial design will help inform both primary and secondary end points and enrich the clinical trial population for potential responders, improving the likelihood of success.

Acknowledgments:
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GEN-27: Identification of GGC repeat expansion in the NOTCH2NLC gene in amyotrophic lateral sclerosis

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Background:
Recently, expanded GGC repeats in the NOTCH2NLC gene have been identified as the pathogenic cause of neuronal intranuclear inclusion disease (NIID)(1). There are several clinical manifestations and pathological changes that are shared between NIID and ALS(2-4).

Objectives:
To determine whether the GGC repeats in the NOTCH2NLC gene contribute to ALS.

Methods:
In this study, 545 ALS patients and 1305 healthy controls from mainland China were recruited. Several pathogenic mutations in known ALS-causative genes (including C9ORF72 and ATXN2) and polynucleotide repeat expansions in NOP56 and AR genes were excluded. Repeat-primed polymerase chain reaction PCR (RP-PCR) and GC-rich PCR were performed to determine the GGC repeat size in NOTCH2NLC. Systematic and targeted clinical evaluations and investigations, including skin biopsy and dynamic electrophysiological studies, were conducted in the genetically affected patients.

Results:
GGC repeat expansion was observed in four patients (numbers of repeats: 44, 54, 96, and 143), accounting for approximately 0.73% (4/545) of all ALS patients. A comparison with 1305 healthy controls revealed that GGC repeat expansion in NOTCH2NLC was associated with ALS (Fisher's exact test, 4/545 vs 0/1305, p=0.007). Compared to patients with the NIID muscle-weakness-dominant subtype, patients with ALS phenotype carrying the abnormal repeat expansion tended to have a severe phenotype and rapid deterioration.

Discussion:
Our results suggest that ALS is a specific phenotype of NIID or that GGC expansion in NOTCH2NLC is a factor that modifies ALS. These findings may help clarify the pathogenic mechanism of ALS and may expand the known clinical spectrum of NIID.

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GEN-28: Identity by descent analysis links SOD1 familial and sporadic ALS cases

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Background:
Approximately 10% of motor neuron disease (MND) cases have a family history of disease, while the remaining cases present as apparently sporadic. Heritability studies suggest a significant genetic component to sporadic MND, and although most sporadic cases have an unknown genetic etiology [1], mutations that are present in familial MND cases have also been found in sporadic cases [2]. This suggests that some sporadic cases may actually be unrecognised familial cases with reduced penetrance. Identifying a familial basis of disease in apparently sporadic MND cases has important genetic counselling implications for immediate relatives, including a 50% chance of inheriting the mutation and a significantly increased chance of developing MND. Identity-by-descent (IBD) analysis detects genomic regions that have been inherited from a common ancestor [3] and is a powerful strategy to uncover a familial basis in apparently sporadic cases.

Objectives:
We sought to determine if Australian ALS families carrying identical SOD1 mutations were distantly related and therefore have a common founder, and if sporadic ALS cases carrying a SOD1 mutation were low-penetrance familial cases.

Methods:
Using TRIBES [4], we performed IBD analysis on 83 Australian familial MND cases from 25 families and three sporadic MND cases, each carrying one of three common SOD1 mutations in Australia (p.I114T, p.V149G and p.E101G). Networks of IBD sharing over SOD1 were generated using isoRelate [5] and mutation dating was performed using the Gamma method [6].

Results:
We identified five unique 350-SNP haplotypes that carry these mutations in our cohort, indicative of five founder events. This included two different haplotypes carrying SOD1 p.I114T, linking familial and sporadic cases. We found that SOD1 p.E101G arose independently in each family that carries this mutation and linked two families carrying SOD1 p.V149G. The age of disease onset differed between cases that carried each SOD1 p.I114T haplotype, while the rate of disease progression differed between cases that carried each SOD1 p.E101G haplotype.

Discussion:
We have shown that IBD analysis is a powerful method to identify distantly related individuals and uncover founder events in individuals with a known ALS mutation. Linking apparently sporadic ALS cases to familial cases can potentially reclassify these cases as low-penetrant familial cases, with significant implications for genetic counselling in these patients and their immediate families.

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GEN-29: Implicating novel genetic variation in ALS through the analysis of disease discordant monozygotic twins

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Background:
The clinical presentation of ALS is highly variable. While disease onset generally occurs between ~50-60 years, ALS may develop as early as the second of life, or as late as >90 years of age. Approximately 10% of cases have a family history (familial ALS) while the remaining 90% are considered sporadic (sporadic ALS) cases. To-date, genetic alterations remain the only established causal and risk factors for ALS. Hundreds of genetic changes from more than 25 genes have been implicated in ALS, making it genetically heterogenous. Despite heritability estimates of 40-60% for all forms of ALS, over 90% of cases have an unknown genetic basis.

Objectives:
Phenotypically discordant monozygotic twins are regarded as a powerful genetic resource. Monozygotic twin pairs discordant for ALS offer a unique opportunity to identify somatic variation between them that contributes to the cause or clinical manifestation of ALS. Using whole genome sequencing (WGS) data from ALS discordant twin/triplet sets (n=5), we aim to uncover novel nucleotide level and/or structural variation contributing to ALS. This includes ALS causal and/or risk variants in twin pairs where one twin is affected by sporadic ALS (n=2), as well as phenotypic modifier variants influencing age of disease onset in twin/triplet sets with a family history of ALS and carrying causal mutations in SOD1 (n=1), C9orf72 (n=1) or an unidentified ALS gene (n=1).

Methods:
Custom bioinformatic analysis pipelines were applied to WGS data to identify putative somatic variation differences between ALS discordant co-twins. Somatic variant calling tools Mutect2 and VarScan2 were used to first independently identify nucleotide level differences, with the results of these two tools then intersected to determine high confidence putative somatic variants. A structural variant call set was generated using the tools Lumpy, Manta and MetaSV, with somatic variation subsequently determined using the GenomeStrip ReciprocalOverlapAnnotator utility, with a reciprocal overlap threshold of <50%. Low fidelity regions of the genome were excluded from analysis, and all putative somatic variation will undergo wet-lab validation. Validated somatic variants will be screened through an additional >700 ALS cases to evaluate their contribution to ALS pathogenesis.

Results:
Bioinformatic analysis has been completed for all twin pairs. The number of putative somatic variants ranges between one to nine for nucleotide level variation, and 105-152 structural variants with no overlap, and a further 0-8 with 0-50% overlap. Wet-lab validation is underway.

Discussion:
This work will implicate novel genes in the cause or risk for ALS, and the modification of age of ALS onset, and further implicate novel pathways or mechanisms in ALS pathogenesis. Each ALS gene identified here will act as a target for functional investigation to tease apart disease mechanisms, and for the development of therapeutics to delay or prevent the onset of ALS.

GEN-30: Novel FUS mutation Y526F and R481Efs48 causing rapidly progressive familial amyotrophic lateral sclerosis

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Background:

FUS gene is one of the most common mutated genes in amyotrophic lateral sclerosis (ALS).

Objectives:

We sequenced for FUS mutations in a cohort of 20 familial ALS and 275 sporadic ALS of Chinese origin.

Methods:

All 15 exons of the FUS gene were sequenced by targeted next-generation sequencing.

Results:

One novel p.Y526F missense mutation, one novel p.R481Efs48 frameshift mutation, and two known missense mutation, p.K510R and p.P525L, in the FUS gene was each detected in one familial ALS proband. One novel FUS p.Q140R variant, and one known FUS frameshift mutation p.R495Efs*33 was each identified in one sporadic ALS case. Another known missense FUS mutation p.R521C were detected in two sporadic ALS patients. The frequency of FUS mutation in our cohort is 20% in familial ALS and 1.5% in sporadic ALS. All patients with FUS mutations had a young onset except the patient with p.Q140R variant. The familial ALS proband carrying the FUS p.Y526F mutation presented with juvenile-onset lower limbs weakness and demonstrated an aggressive course, with respiratory muscles involvement six months after onset. The other patients in the family all had limbs weakness and died 1-2 years after disease onset. The familial ALS proband carrying the FUS p.R481Efs48 mutation also presented with juvenile-onset lower motor neuron predominant ALS and demonstrated an aggressive course, his mother had limbs weakness at age 28 and died 2 years after disease onset. The familial ALS proband carrying the FUS p.P525L mutation presented with bulbar-onset ALS and died of respiratory failure 7 months after onset. The two patients carrying p.R521C mutation presented with weakness of lower limbs at the age of 30 and 33, respectively. One developed lower motor neuron predominant ALS and another developed ALS with dropped head syndrome. They died at 18 and 17 months after disease onset, respectively.

Discussion:

Our results strengthen that FUS mutations are the most frequent genetic causes of young-onset aggressive ALS. Genetic testing of the FUS gene should be performed in early-onset ALS patients especially those with a rapid progression.

GEN-31: Novel STMN2 Variant Linked to Sporadic Amyotrophic Lateral Sclerosis Risk and Phenotype

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Objective:
There is a critical need to establish new genetic markers that may explain differences in the complex phenotypes and pathogenicity of amyotrophic lateral sclerosis. This study identified a CA repeat polymorphism in the Stathmin-2 gene and investigated its association with sporadic (sALS) disease risk, age-of onset and survival.

Methods:
A novel variable length dinucleotide CA repeat was systematically analyzed using polymerase chain reaction, polyacrylamide fractionation, Sanger sequencing and high throughput capillary separation for genotyping. Stathmin-2 expression was measured in control and sALS patient olfactory neurosphere-derived cells using RT-PCR, and further evaluated in control and sALS patient laser captured motor neurons with accompanying RNA sequencing data.

Results:
In a North American sALS cohort (n = 321), long/long genotypes of the CA variant were associated with disease risk (p = 0.042), especially when they included a 24 CA allele (p = 0.0023). In addition, carriage of long alleles was associated with a 7.5-year earlier age-of-onset (p = 0.039), and survival duration was significantly reduced in the bulbar-onset subgroup of patients with long/long 24 CA genotypes (p = 0.006). In an Australian Caucasian longitudinal follow up sALS cohort (n = 67) there was a significant reduction in ALS functional rating scale (ALSFRS) in carriers of L/L genotypes (p = 0.034). Expression of Stathmin-2 (STMN2) was reduced in sporadic patient olfactory neurosphere-derived cells, independent of Transactive response DNA binding protein-43kDa mRNA expression. Additionally, laser captured spinal motor neurons from sALS patients and controls with short/long genotypes appeared to display increased STMN2 expression compared to long/long genotypes, however this did not reach statistical significance.

Discussion:
This is the first report of risk and disease-modifying effects of a non-coding variant in STMN2 in sALS. Further studies are required to validate the present findings in additional cohorts, and to investigate the potential value of the STMN2 CA repeat as a genetic marker for patient stratification and enrichment in clinical trials.

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GEN-32: Role of hnRNPA1 in an Italian ALS Population-Based Cohort

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Background:
Mutations in the prion-like domain of the gene encoding the hnRNPA1 protein have been implied in Amyotrophic Lateral Sclerosis (ALS) pathogenesis. These mutations have been reported in ALS patients: however, because of the lack of clinical evidence, the contribution of hnRNPA1 to ALS is inconclusive and the phenotype of patients with mutated hnRNPA1 is still unclear.

Objectives:
To unravel the role of heterogeneous nuclear ribonucleoproteins (hnRNP) in ALS, we examined hnRNPA1 rare variants in an ALS cohort from Northern Italy. We sought to determine the prevalence of HNRNPA1 mutations in ALS patients and their associated clinical phenotype.

Methods:
We identified variants in hnRNPA1 gene through whole-genome sequencing of 957 individuals with sporadic and familial ALS from the Piemonte ALS Register (PARALS) and 677 control subjects. We performed a gene-based rare variants analysis and then assessed the clinical characteristics of the patients who carry the candidate disease-associated variants.

Results:
We detect an enrichment of hnRNPA1 rare variants in ALS patients (p-value 0.034). We found 5 (0.5%) individuals carrying 5 distinct missense variants that were absent in the control population. Four of these variants are located in the prion-like domain of the gene: the variant c.C666G (p.F222L) in exon 6 and the variants c.G824T (p.G275V), c.C876G (p.N292K) and G883A (p.G295R) in exon 8. All cases were sporadic. Limb onset occurred in all five patients. Mean age of onset was 58.0 years. The mean rate of decline for ALS-FRS was 0.37 points/months; two patients showed a late involvement of bulbar (29.0 months) and respiratory functions (39.5 months). None of the patients showed cognitive impairment. hnRNPA1 variants were associated with a longer survival (HR 13.7, 95% C.I. 4.78-39.5, $p < 0.001$) than non-mutated ALS patients from the PARALS.

Conclusions:
We describe the correlation between the hnRNPA1 genotype and ALS phenotype. Our data demonstrate that mutations in hnRNPA1 are a low-frequency cause of disease in our cohort and they define a relatively uniform, slow-progressive subset of ALS.

GEN-33: The use of ExpansionHunter and ExpansionHunter Denovo in an Australian ALS cohort detects known and novel repeat expansions

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Background:
Structural variants, in particular repeat expansions, represent a significant source of genetic variation in the human genome and are recognised to contribute to neurodegenerative diseases. Expansions identified to contribute to ALS include the most well-known C9orf72 hexanucleotide repeat and three more recently identified ALS risk regions in ATXN1, ATXN2 and NIPA1. Low-throughput techniques have previously limited the genome-wide genotyping of repetitive regions, however, the advent of computational tools capable of predicting expansions from short-read whole genome sequencing, combined with large population reference data, now provides a platform to permit initial analyses to detect repeat expansions contributing to disease.

Objective:
To examine whole-genome sequences in ALS cases and controls to profile known and novel repeat expansions in ALS cases compared to controls.

Methods:
To identify known and novel expansion regions associated with ALS cases, ExpansionHunter (EH) and ExpansionHunter Denovo (EHdn) were applied to a preliminary discovery cohort of Australian ALS cases (66) and controls (40). Novel candidate loci (EHdn outlier analysis via the locus method) were prioritised for follow-up with high z-scores, short motifs, present in >1 ALS case. Candidate expansions and known regions were screened with the complimentary tool EH to provide the number of repeats in each individual for each specified locus. They were confirmed by at least molecular technique before being analysed in 657 European controls, age >50 years (TOPMed) to identify if the expansion was enriched in cases.

Results:
Known expansion loci were identified by EH and molecularly validated with high precision (>96%) in C9orf72, ATXN2, and ATXN1. EHdn detected many thousands of loci with subsequent filtering identifying four candidate regions, one of which was both molecularly validated and enriched in cases compared to controls. A pentanucleotide intronic expansion (>30 repeats, vs. expected 9-17 repeats) in a gene previously not linked to ALS was identified in 5/66 cases compared to 5/698 controls (p=0.003, Fisher's exact test).

Discussion/Conclusion:
Our preliminary analysis characterised four known expansion regions in an Australian cohort and surprisingly, detected one novel region potentially contributing to ALS risk on chromosome 1. Replication and functional validation are now needed as the gene has previously been upregulated in SOD1G93A ALS mouse model and may implicate a role in the immune system as it is involved in NF-κB activation. Our whole genome sequencing on a small cohort highlights both the unexplored avenue of structural variation in ALS and the need for large control data and validation. Additional replication in cases is currently being carried out.