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BOOK OF ABSTRACTS

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Poster Session 1: Wednesday 20th June, 18:00 - 19:30

Entrance Hall:

A01 Hot-spot KIF5A mutations cause familial ALS

David Brenner* (1), Rüstem Yilmaz (1), Kathrin Müller (1), Torsten Grehl (2), Susanne Petri (3), Thomas Meyer (4), Julian Grosskreutz (5), Patrick Weydt (1, 6), Wolfgang Ruf (1), Christoph Neuwirth (7), Markus Weber (7), Susana Pinto (8, 9), Kristl G. Claeys (10, 11, 12), Berthold Schrank (13), Berit Jordan (14), Antje Knehr (1), Kornelia Günther (1), Annemarie Hübers (1), Daniel Zeller (15), The German ALS network MND-NET, Christian Kubisch (16, 17), Sibylle Jablonka (18), Michael Sendtner (18), Thomas Klopstock (19), Mamede de Carvalho (8, 20), Anne Sperfeld (14), Guntram Borck (16), Alexander E. Volk (16, 17), Johannes Dorst (1), Joachim Weis (10), Markus Otto (1), Joachim Schuster (1), Kelly del Tredici (1), Heiko Braak (1), Karin M. Danzer (1), Axel Freischmidt (1), Thomas Meitinger (21), Tim M. Strom (21), Albert C. Ludolph (1), Peter M. Andersen (1, 9), and Jochen H. Weishaupt (1)

Heterozygous missense mutations in the N-terminal motor or coiled-coil domains of the kinesin family member 5A (KIF5A) gene cause monogenic spastic paraplegia (HSP10) and Charcot-Marie-Tooth disease type 2 (CMT2). Moreover, heterozygous de novo frame-shift mutations in the C-terminal domain of KIF5A are associated with neonatal intractable myoclonus, a neurodevelopmental syndrome. These findings, together with the observation that many of the disease genes associated with amyotrophic lateral sclerosis disrupt cytoskeletal function and intracellular transport, led us to hypothesize that mutations in KIF5A are also a cause of amyotrophic lateral sclerosis. Using whole exome sequencing followed by rare variant analysis of 426 patients with familial amyotrophic lateral sclerosis and 6137 control subjects, we detected an enrichment of KIF5A splice-site mutations in amyotrophic lateral sclerosis (2/426 compared to 0/6137 in controls; $P = 4.2 \times 10–3$), both located in a hot-spot in the C-terminus of the protein and predicted to affect splicing exon 27. We additionally show co-segregation with amyotrophic lateral sclerosis of two canonical splice-site mutations in two families. Investigation of lymphoblast cell lines from patients with KIF5A splice-site mutations revealed the loss of mutant RNA expression and suggested haploinsufficiency as the most probable underlying molecular mechanism. Furthermore, mRNA sequencing of a rare non-synonymous missense mutation (predicting p.Arg1007Gly) located in the C-terminus of the protein shortly upstream of the splice donor of exon 27 revealed defective KIF5A pre-mRNA splicing in respective patient-derived cell lines owing to abrogation of the donor site. Finally, the non-synonymous single nucleotide variant

rs113247976 (minor allele frequency = 1.00% in controls, n = 6137), also located in the C-terminal region [p.(Pro986Leu) in exon 26], was significantly enriched in familial amyotrophic lateral sclerosis patients (minor allele frequency = 3.40%; P = 1.28 × 10−7). Our study demonstrates that mutations located specifically in a C-terminal hotspot of KIF5A can cause a classical amyotrophic lateral sclerosis phenotype, and underline the involvement of intracellular transport processes in amyotrophic lateral sclerosis pathogenesis.

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A02 Alterations of C9orf72, SOD1, TARDBP, FUS and UBQLN2 genes with amyotrophic lateral sclerosis

Vildan Çiftçi 1, Türker Bilgen 2, Şule Darbaş 1, Yunus Arıkan 1, Hilmi Uysal 3*, Sibel Berker Karaüzüm 1

Objective: The aim of this study is to determine existence and frequency of the genomic alterations in C9orf72, SOD1, TARDBP, FUS and UBQLN2 genes which are frequently observed in familial(fALS) and sporadic(sALS) ALS diagnosed cases. Materials-Methods: Genomic DNA was isolated from peripheral blood in 12 fALS and 18 sALS. C9orf72 gene expansion was checked by triplet-primer PCR amplification and fragment analysis. All sequences encoding the SOD1, TARDBP, FUS and UBQLN2 genes were screened by DNA sequencing. Results: In intron1 of C9orf72 gene in 2 sALS cases c.-45+162_-45+163insGGGGCC heterozygote change was detected. In SOD1 gene in 2 fALS and 5 sALS heterozygote and in 1 sALS homozygote c.72+133C>T change in intron1; in 1 fALS novel c.169+41C>A heterozygote change in intron 2; in 3 fALS c.239+34A>C heterozygote change in intron 3 was found. In intron 5 of TARDBP gene in 6 cases c.714+67_714+68insG homozygote change was detected. In FUS gene mutation screening revealed c.147C>A change in exon3 with heterozygosity in 3 sALS, in exon4 c.291C>T change with heterozygosity in 5 fALS and 8 sALS and with homozygousity in 4 fALS and 8 sALS; also in intron 11 novel c.1067-61T>C heterozygote in 3 sALS and 1 fALS. In UBQLN2 gene 1 sALS novel c.1371G>C heterozygote change was determinated. Conclusion: C9orf72 hexanucleotide repeat expansion was observed 6,6% in 32 cases as known pathogenic mutation. In this study, three novel changes have been identified. Clinical significance of these changes is not yet known. The existence of variation in FUS gene in all cases supports that this gene is highly polymorphic. Keywords: ALS, sporadic ALS, familial ALS, genomic alterations

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A03 Novel ALS-associated mutations in the ARPP21 gene cause abnormal protein aggregation and altered neuronal morphology

Chun Hao Wong (1)(*), Simon D Topp (1), Youn-Bok Lee (1), Sarah Mueller (1), Graham Cocks (1), Bradley N Smith (1), Nicola Ticozzi (2), John Landers (3), Christopher E Shaw (1)

Background ALS is a highly heterogeneous neurodegenerative disease in which multiple, rare genetic causes may contribute. Here, we report a new disease-associated gene ARPP21 identified through whole-exome sequencing in a cohort of familial ALS cases. Two novel variants are shared by several unrelated index cases with no mutations in currently known ALS genes. To validate the involvement of ARPP21 in ALS, we have screened the gene extensively and studied the identified variants in cellular models. Materials and Methods Direct sequencing was performed on over 2000 ALS cases and 1000 controls of UK, US and Italian origin. Disease modelling was performed in HEK239T, SH-SY5Y and primary rat cortical neurons. Results Both variants linked with familial ALS (p.P563L; p.P747L) are replicated in a sporadic ALS cohort. The p.Proline563 residue is found to be substituted to glutamine in two sporadic cases of UK and Italian origin. These mutations are absent in both public databases and local control cohorts. Immunohistochemistry against the endogenous ARPP21 protein in human post-mortem cerebellum and spinal cord tissues suggests that it is a predominantly cytosolic phosphoprotein that is highly enriched in the neuronal population. Functional assays indicate that the ALS-associated mutations could cause disease hallmarks including enhanced detergent-insoluble aggregates, dysregulation in proteasome degradation, decreased neurite outgrowth, and altered dendritic morphology. A proportion of mutant ARPP21 aggregates co-localise with cytoplasmic TDP-43 granules, suggesting a toxic gain-of-function mechanism. Conclusions Mutation frequencies of ARPP21 in current study are approximately 1.6% in familial cases and 0.3% in sporadic cases. Cellular studies of ARPP21 mutations have recapitulated pathological hallmarks of ALS. By combining the genetics and follow-up functional assays; we have identified a novel disease-associated gene that has not been previously linked with neurodegeneration.

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A04 TBK1 variants and sporadic ALS: Looking for accomplices

Giuseppe Marangi* (1), Serena Lattante (1), Amelia Conte (2), Giulia Bisogni (2), Daniela Bernardo (2), Paolo Doronzio (1), Nilo Riva (3), Christian Lunetta (4), Marcella Zollino (1), Mario Sabatelli (2)

Variants in TBK1 are responsible for a high proportion of sporadic ALS cases. In the present study we analysed variants in TBK1 estracted by a targeted sequencing of a panel of 30 genes in a group of 294 Italian ALS patients. We identified 5 different TBK1 variants in 5 sporadic cases, resulting in a frequency of 1.7 % , similar to that found in previously described cohorts. Two patients had missense variants (p.R357Q and p.R724C), one patient had a small deletion (p.616_617del) and two had frameshift variants (p.V481fs*1, p.S680fs*1). We tested TBK1 expression in primary fibroblasts cultures by western blot and we found that the protein levels were significantly decreased in patients with frameshift and deletion variants and in the patient with the p.R357Q variant. Since an oligogenic model may explain the sporadic occurrence of ALS in patients with a known genetic defect, we looked for additional mutations in other ALS genes. The patient with the p.S680fs*1 carried a concomitant missense variant (p.E408A) in exon 12 of OPTN and showed a peculiar phenotype characterized by striking bulbo-spinal spasticity and overt signs of FTD. Two additional patients carried a concomitant variant in the 3'UTR region of FUS gene. Based on these observation we studied an independent group of 7 TBK1 mutated patients recently reported (Pozzi et al 2017) and we found another variant in the 3'UTR region of FUS in one patient. In all Italian patients carrying TBK1 variants described up to now the disease occurred sporadically. This observation suggests that TBK1 belongs to the category of genes conferring a significantly increased risk, but not sufficient to cause disease, most likely. Olygogenic architecture of ALS is observed in a small proportion of patients harbouring mutations in such ALS-related genes. The presence of a second variant in a high proportion (1/3) of our TBK1-patients is a striking and novel observation. Previous genetic and biochemical studies consistently indicated that mutation in the regulatory region of FUS are associated with ALS and increase FUS expression dramatically. Since autophagy is critical to managing the burden of misfolded and toxic proteins, it is not unexpected that perturbed autophagy induced by TBK1 mutations can exacerbate the consequences of overexpression of wild-type FUS. Further studies are needed to clarify the hypothesis that variants in the 3'UTR region of FUS act as veiled and perhaps customary acc

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A05 Repeat expansion detection from whole-genome sequence data of Project MinE

Joke J.F.A. van Vugt (1*), Egor Dolzhenko (2), Richard J. Shaw (3,4), Maarten Kooyman (5), Gijs H.P. Tazelaar (1), Michael A. van Es (1), Project MinE ALS Sequencing Consortium, Leonard H. van den Berg (1), Michael A. Eberle (2) and Jan H. Veldink (1)

Identifying changes in short tandem repeats (STRs) that are a risk factor for amyotrophic lateral sclerosis (ALS) is challenging for short-read (100-150 bp) whole-genome sequencing (WGS) data. Tools like ExpansionHunter(1) and HipSTR(2) exist that can genotype STRs in the human genome from WGS data. These tools require the exact locus and motif of an STR as input. HipSTR comes with ~1.7 million STR loci but can only identify changes in STRs that stay shorter than the read length. STRs longer than the read length are not reported by HipSTR, which can cause possible ALS-related repeat expansions to be missed. ExpansionHunter can genotype STRs even if the repeat is longer than the read length and comes with over 209 thousand STR loci. A tool that is being developed by Illumina can detect STRs longer than the read length without the need of a genome locus or motif as input. Potential ALS-related STR loci that followed from this untargeted tool are used as input in ExpansionHunter and genotyped accordingly. With the combined analysis of untargeted and targeted STR genotyping tools we aim to identify unknown ALS-related STRs from WGS data. These findings will be validated by means of replication in WGS data and wet lab techniques. Known ALS-related STRs like the ones in the NIPA1, ATXN1 and ATXN2 genes, for which we have PCR and Sanger sequencing results, help us to establish a measure for the genotyping quality control. (1) Dolzhenko and Van Vugt et al. (2017) Genome Research 27(11):1895-1903, doi:10.1101/gr.225672.117 (2) Willems et al. (2017) Nature Methods 14(6):590-592, doi:10.1038/nmeth.4267

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A06 Whole-genome variant analysis of Spanish monozygotic twins discordant for ALS disease

Gerardo Alonso-Munguía (1)*, Yolanda Campos (1), Jesus Mora (2), Teresa Salas (3), Victoria López-Alonso (4)

Genome sequencing has contributed to the acceleration in the discovery of genetic risk factors for sporadic and familial patients with Amyotrophic lateral sclerosis (ALS). Over the last decade, identification of mutations in about 20 genes predisposing to this disorder has provided the means to better understand their pathophysiology. In this poster we show the genome analysis of a couple of Spanish monozygotic twins with and without ALS, study supported by Fundela (http://www.fundela.es/) as part of the Project MinE. Whole genome sequence variant discovery was done with the Broad Institute GATK tool, and annotation and filtering of the variant call format (VCF) files were carried out using Bystro web application. We analyze single nucleotide variations, insertions and deletions in genes and intergenic regions that could be significantly involved in gene regulation and function. Moreover, we will evaluate signal and metabolic pathways that could be affected by the detected changes found in the genomes comparison. The study aimed to identify the specific genetic variants that could be associated to contribute to the knowledge of the genetic of ALS.

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A07 Additional SQSTM1 mutations in ALS patients

Rustem Yilmaz* (1), Kathrin Müller (1), David Brenner (1), Albert Ludolph (1), Peter Andersen (1,2), Jochen Weishaupt (1)

Amyotrophic lateral sclerosis is a genetically highly heterogeneous neurodegenerative disorder, for which more than 25 genes have been reported as causative up to date. Grouping the genes linked with ALS according to their physiological functions implicates few main biological pathways. For example, several studies reported mutations in TBK1, OPTN, VCP, SQSTM1 and UBQLN2, genes encoding proteins involved in autophagy, as causative for ALS. SQSTM1 was first identified by candidate gene analysis due to its functional role in protein quality control. It codes for p62, a multifunctional protein which functions in protein degradation both through proteasome and autophagy. Both p62 and optineurin are direct interaction partners and substrates of the recently discovered ALS gene TBK1, and form the core of a genetic and functional network that may connect autophagy with ALS. We set out to review the current evidence for SQSTM1 as an ALS disease gene and screened for SQSTM1 mutations in 484 FALS patients mainly with German or Nordic origin by whole exome sequencing (WES), followed by segregation analysis. We reported eight novel and five previously reported rare variants in SQSTM1, and discuss the current evidence for SQSTM1 as a Mendelian disease gene for ALS.

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A08 Analysis of ascertainment bias in ALS

Puja R. Mehta (1)*, Ashley Jones (1), Sarah Martin (1), Alfredo Iacoangeli (1), Ammar Al-Chalabi (1)

Background: Familial ALS has an age of onset (AOO) about 10 years younger than apparently sporadic ALS. It is unclear if this is due to the presence of ALS genes, or because a family history increases awareness of patients and doctors, leading to more rapid diagnosis (ascertainment bias). We therefore investigated the relative contributions of genes and awareness to the reduced AOO. Methods: 941 people of European ancestry with a diagnosis of ALS were included. There were 841 with apparently sporadic ALS and 100 with familial ALS. Samples were obtained from the UK National DNA Bank for Motor Neuron Disease Research and sequenced using MiSeq technology. A panel of 13 ALS genes was tested (ANG, C9ORF72, DAO, DCTN1, FUS, OPTN, PFN1, SOD1, SQSTM1, TARDBP, UBQLN2, VAPB, and VCP). The median time between symptom onset and diagnosis was compared between apparently sporadic ALS and familial ALS to confirm ascertainment bias. Mean AOO was compared between sporadic ALS cases with and without a pathogenic ALS gene variant to determine the effect of ALS genes on AOO, and between genetic sporadic ALS and familial ALS to determine the effect of family history and, therefore, ascertainment bias, on AOO. Results: There were 95 people with apparently sporadic ALS carrying a presumed pathogenic gene variant, and 746 without. Median time between symptom onset and diagnosis was 3 months shorter for familial ALS than apparently sporadic ALS ($p = 0.012$). Mean AOO in familial ALS was 5.3 years younger than for apparently sporadic ALS ($p = 6.0 \times 10(-5)$, 95% CI of the age difference 2.8-7.8 years). Mean AOO of genetic sporadic ALS was 2.9 years younger than non-genetic sporadic ALS ($p = 0.011$, 95% CI of the age difference 0.68-5.2 years). There was no difference between the mean AOO in genetic sporadic ALS and familial ALS ($p = 0.097$). Conclusions: The quicker time from symptom onset to diagnosis in familial ALS compared to apparently sporadic ALS confirms ascertainment bias. The younger AOO of patients with familial ALS is not explained by ascertainment bias, but is a genetic effect.

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A09 Whole exome sequencing identifies novel and recurrent variants in Hungarian patients with ALS

Kornélia Tripolszki (1)*, Dóra Nagy (1), Zsófia F. Nagy (1), József I. Engelhardt (2), Péter Klivényi (2), Márta Széll (1)

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Genetic factors play a key role in ALS and uncovering its genetic background may bring us closer to fully understand its pathomechanism. Therefore, the aim was to identify rare damaging variants in major and minor genes involved in pathways annotated to ALS and in genes of other neurogenetic disorders. Patients and methods: 21 Hungarian patients with sporadic ALS were enrolled in the study. They all fulfilled the revised El Escorial and the Awaji-shima criteria for ALS. Prior to whole exome sequencing, the patients were prescreened for 7 major ALS genes: C9ORF72 repeat expansion, SOD1, ANG, FUS, SETX, TARDBP and UBQLN2. Exome sequencing was carried out by using Illumina NextSeq sequencer and data analysis was performed according to the best practices to identify single nucleotide variants and small insertions/deletions. Variant filtering was performed to identify damaging variants in ALS "priority" genes (n=32), ALS "candidate" genes (n=98) and other neurogenetic disease genes (n=125). The detected variants were confirmed by Sanger sequencing. Results: In ALS "priority" genes, exome sequencing revealed a novel non-synonymous variant (T338I) in the NEFH gene that encodes neurofilament heavy polypeptide; a novel non-synonymous variant (P11S) in the MATR3 gene that is specific to the matrin-3 isoform 1; a previously described pathogenic nonsense mutation (G1177X) in the alsin (ALS2) gene that leads to premature stop codon and may affect endosomal and vesicle transport; a rare variant (R2034Q) in the SPG11 gene with uncertain significance; and finally a known variant (R261H) in the NEK1 gene, encoding NIMA-related kinase-1, that has recently been associated with ALS in the Caucasian population. Further novel and rare recurrent variants have been detected in 10 ALS "candidate" genes and in 7 other neurogenetic disease genes. None of the detected variants were present in healthy Hungarian controls. Conclusion: Potentially disease-causing variants in ALS "priority" genes have been detected in 23% (5/21) of this sporadic cohort. While the disease causing role of several mutations identified in this study has been previously well-established, other variants may show reduced penetrance or may be rare benign variants. Our study provides further insight into the genetic etiology of this heterogenous disease. Funding: Hungarian Brain Research Program (Grant No. KTIA_13_NAP-A-II/15)

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A10 Testing for synergy between loss and gain of FUS function in causing motor neuron degeneration

Sanjuan-Ruiz I (1), Myers B (2), McAlonis-Downes M (2) , Cleveland DW (2), Lagier-Tourenne C (3), Da Cruz S (2) & Dupuis L (1)

FUS is an RNA-binding protein involved in the regulation and transport of proteins from the nucleus to the cytoplasm. Mutations in this gene have been linked to ALS with early onset and short life expectancy. Mutations in the FUS gene disrupt FUS nuclear localization and the most severe FUS mutations lead to the C-terminal truncation of the protein thereby deleting the nuclear localization sequence. The analysis of the pathophysiological mechanisms of FUS-ALS is complicated by the severe toxicity of FUS overexpression, and the tight mechanisms of autoregulation of FUS protein levels. To overcome these issues, we have generated and characterized conditional knock-in mice expressing mislocalized cytoplasmic FUS (1,2). Using these mice, we have shown that complete FUS cytoplasmic mislocalization leads to motor neuron degeneration, while loss of FUS does not, thus demonstrating that the full toxicity mediated by this mutant truncated FUS requires it to be present in the cytoplasm. To determine whether loss of nuclear FUS contributes to motor neuron degeneration, we crossed our knock-in mice to transgenic mice expressing the human FUS gene (either wild type or carrying an ALS-linked R521H mutation). Both wild type and ALS-linked mutant of FUS rescue the lethality of homozygous knock-in mice expressing cytoplasmically localized FUS. Histological and molecular characterization of these compound transgenic mice are ongoing. These studies have important consequences for potential therapeutics targeting the FUS gene. [1] Scekic-Zahirovic J, Sendscheid O, El Oussini H et al., Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss, EMBO J, 2016, 35, (1077-1097) [2] Scekic-Zahirovic J, El Oussini H et al., Motor neuron intrinsic and extrinsic mechanisms contribute to the pathogenesis of FUS-associated amyotrophic lateral sclerosis, Acta Neuropathol, 2017, 133 (887-906)

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A11 Investigating TBK1-dependent signalling pathways in Amyotrophic Lateral Sclerosis

Maria Davies (1*), Mark O. Collins (1)

Tumor necrosis factor receptor-associated factor NF-KB (TANK) binding kinase 1 (TBK1) was recently identified as a novel amyotrophic lateral sclerosis (ALS) gene in numerous independent human genetic studies. TBK1 is a non-canonical IkB kinase, playing a role in autophagy and the innate immune response. Over 145 TBK1 mutations along the entire protein length, predicted to cause TBK1 loss of function, have been found in ALS patients and patients with ALS-frontotemporal dementia (FTD). TBK1 mutations are found in approximately 3.6% of all ALS, ALS-FTD and FTD cases, but exactly how TBK1 mutations are contributing towards disease is unclear. It is hypothesised that TBK1 mutations in ALS patients contribute to disease pathology due to impaired autophagy, and in particular mitophagy. These two processes are crucial for homeostasis and are involved in neurodegenerative diseases, as well as basal autophagy levels being high in neurons. Given that aggregate formation is a pathological feature of ALS, removal of these aggregates by autophagy is of particular relevance in this disease. The main aim of this work is to investigate pathways regulated by TBK1 in a neuronal-like setting, and to elucidate how TBK1 mutations contribute to the pathogenesis of ALS. Conditions for autophagy induction have been established in NSC-34 cells and the regulation of autophagy by TBK1 is being investigated in this system. TBK1 regulates autophagy through two main processes; the phosphorylation of autophagy adaptors sequestosome/p62 and optineurin, as well as regulating autophagosome maturation into autolysosomes. We are investigating the role of TBK1 using small molecule inhibition and knockdown studies combined with biochemical, proteomic and cell biological approaches. The TBK1 signalling network will be expanded to further the knowledge of TBK1 substrates, specifically those involved in the autophagic setting.

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A12 MIF inhibits the formation and toxicity of misfolded SOD1 amyloid aggregates: Implications for familial ALS

Argueti. S(*) Shvil N (1), Banerjee V (2), Zoltsman G (1), Shani T (1), Kahn J (1), Abu-Hamad S (1), Papo N (2,3), Engel S (3,4), Bernhagen J (5), Israelson A (6,7)

Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease caused by the progressive loss of motor neurons in the brain and spinal cord. It has been suggested that toxicity of mutant SOD1 results from its misfolding, however, it is yet unclear why misfolded SOD1 accumulates specifically within motor neurons. We recently demonstrated that macrophage migration inhibitory factor (MIF)-a multifunctional protein with cytokine/chemokine activity and cytosolic chaperone-like properties-inhibits the accumulation of misfolded SOD1. Here, we show that MIF inhibits mutant SOD1 nuclear clearance when overexpressed in motor neuron-like NSC-34 cells. In addition, MIF alters the typical SOD1 amyloid aggregation pathway in vitro, and, instead, promotes the formation of disordered aggregates, as measured by Thioflavin T (ThT) assay and transmission electron microscopy (TEM) imaging. Moreover, we report that MIF reduces the toxicity of misfolded SOD1 by directly interacting with it, and that the chaperone function and protective effect of MIF in neuronal cultures do not require its intrinsic catalytic activities. Importantly, we report that the locked-trimeric MIFN110C mutant, which exhibits strongly impaired CD74-mediated cytokine functions, has strong chaperone activity, dissociating, for the first time, these two cellular functions. Altogether, our study implicates MIF as a potential therapeutic candidate in the treatment of ALS.

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A13 Role of the calcium-activated chloride channel TMEM16F in amyotrophic lateral sclerosis

Claire Soulard (1)(*), Céline Salsac (1), Kévin Mouzat (2), Cécile Hilaire (1), Serge Lumbroso (2), Cédric Raoul (1), Frédérique Scamps (1)

Amyotrophic lateral sclerosis (ALS) is fatal incurable neurodegenerative disease characterized by the selective loss of motoneurons in the spinal cord, brainstem, and motor cortex. Among the cellular mechanisms leading to motoneuron degeneration, dysregulation of both Ca2+ homeostasis and electrical activity plays a central role in the progression of the disease. In presymptomatic and early symptomatic stages of the disease, changes in both intrinsic and synaptic motoneuron electrical activity have been proposed to contribute to neurodegeneration. To date, targeting electrical activity, through the glutamate release inhibitor riluzol, has been proven to provide the best results on ALS progression in human. Altogether, these data confirm that electrical activity is a major contributor to ALS progression that needs continuous investigations to provide more efficient therapeutic means. Ca2+ is a major regulator of electrical activity and among its numerous cellular functions as a signaling molecule, Ca2+ controls the expression of ionic channels such as Ca2+ activated chloride channels (CaCCs) that we have shown to be expressed in spinal motoneurons. In addition to the Ca2+ dependent activation of CaCC, the hallmark of Cl- channels is that, depending on the pathophysiologic context, inhibitory or excitatory effects on electrical activity are anticipated due to their dependence towards the expression of cation-chloride cotransporter. Given the importance of calcium and chloride homeostasis in neuronal function and the role of excitability in ALS, we analyze the role of CaCC expressed in spinal motoneurons on ALS progression. We have identified TMEM16F as the protein responsible for the Ca2+-activated chloride channel expressed in spinal motoneurons, in close apposition with C-bouton synapses and at the neuromuscular junction. We also show that TMEM16F is involved in the maintenance of muscle performance. Moreover, analysis of TMEM16F expression during ALS progression indicates a decrease of TMEM16F protein level whereas motoneurons still receive cholinergic inputs from C-boutons. Our results question whether loss of TMEM16F expression is a neurodegenerative effect of ALS or a neuroprotective compensatory mechanism to delay denervation and motoneuron death? To address this question, we are currently investigating whether TMEM16F deficiency in SOD1G93A mice influences the progression of the disease.

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A14 Autophagy interacts with TDP-43 function

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TAR DNA binding protein (TDP-43) is one of the components of neuronal aggregates in sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). We have applied a simple quantitative method to evaluate TARDBP splicing function to spinal cord, brainstem, motor cortex, and occipital cortex samples from ALS (n=8) cases compared to age- and gender-matched control (n=17). Then, we quantified the abundance of TDP-43-spliced cryptic exons present in autophagy related 4B cysteine peptidase (ATG4B) mRNA. Results of these analyses demonstrated that the loss of this TDP-43 function in spinal cord, brainstem, motor cortex, and occipital cortex differentiated ALS from controls (area under the curve of ROC: 0.85). Significant correlations were also observed between cryptic exon levels, age, disease duration, and aberrant mRNA levels. To test if TDP-43 function in splicing is relevant in ATG4B major function (autophagy) we downregulated TDP-43 expression in HeLa and in human neural tissue cells, demonstrating that TDP-43 is required for maintaining the expression of ATG4B. ATG4B overexpression alone is sufficient to completely prevent the increase of SQSTM1 -a hallmark of defective autophagy- induced by TDP-43 downregulation in human neural tissue cells and in cell lines. Autophagy induction by amino acid deprivation in TDP-43 knockdown cells is able to increase remaining TDP-43 function, overall leading to increased ATG4B mRNA and decreased levels of cryptic exon on its mRNA. Thus, the present findings shows that TDP-43 acts synergistically with authophagy to enhance cryptic exon splicing function on key mRNA and constitute a novel pathway for neurodegeneration in ALS, where loss of TDP-43 could impair autophagy, further sensitising cells to noxious stimuli.

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A15 TDP-43 protein aggregation in Amyotrophic Lateral Sclerosis: a role for the post-translational modification SUMOylation

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Amyotrophic Lateral Sclerosis (ALS) is an adult neurodegenerative disease affecting motor neurons. The cytoplasm of the degenerating motor neurons present protein aggregates predominantly composed of TDP-43. The mechanisms leading to the formation of these TDP-43+F16 positive aggregates are still poorly understood. We previously showed that the post-translational modification SUMOylation is involved in aggregate formation in a model overexpressing mutant SOD1, another protein mutated in ALS. The present study concerns the role of SUMOylation pathway in the formation of TDP-43 aggregates. TDP-43 (Tar DNA Binding Protein) is a nuclear protein containing a unique putative SUMOylation site (Lysine 136). We first overexpressed wild-type GFP-TDP-43 or GFP-TDP- 43K136R mutated for this SUMOylation site. Cells overexpressing GFP-TDP43WT showed aggregates in the cytoplasm, as expected. Interestingly, cells overexpressing GFP-TDP-43K136R presented nuclear aggregates. This mutant was also associated with cells displaying longer neurites and a reduced toxicity caused by an over-expression of TDP-43WT . We next studied the effect of anacardic acid (AA), an inhibitor of SUMOylation pathway, on neuronal NSC34 cultures overexpressing TDP-43WT . Preliminary results showed a reduction of aggregates in cells treated with 25 Î¼M of AA. These results suggest a role for the SUMOylation pathway in the regulation of TDP-43 nucleo-cytoplasmic transport and reinforce the idea of an implication of this pathway in the formation of cytoplasmic protein aggregates in ALS (Maurel et al., 2018). Thus, this pathway consists in an attractive therapeutic target and AA a molecule of interest for future work in in vivo models of ALS.

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A16 Mechanisms of paraspeckle hyper-assembly in ALS

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Background. Paraspeckles are nuclear bodies assembled on NEAT1_2, the longer isoform of a lncRNA NEAT1. There is an unusually strong association between ALS pathogenesis and paraspeckles. At least seven paraspeckle proteins, including TDP-43, are genetically linked to ALS. Furthermore, enhanced paraspeckle assembly in spinal neurons in sporadic ALS was reported. However, the mechanistic basis for this association is completely obscure. Objectives. In present study we aimed to dissect mechanisms behind paraspeckle hyper-assembly in ALS. Methods. NEAT1 levels and the presence of paraspeckles were examined in the spinal cord of ALS patients with diverse aetiology using RNA-FISH, RNAscope ISH and qRT-PCR. A range of cell models including hES-derived motor neurons were used for mechanistic studies. Results. We show that paraspeckle formation in the spinal cord is a hallmark of both sporadic and familial ALS, including cases with C9ORF72 and TARDBP mutations. TDP-43 pathology is typical for ~90% of ALS cases, and we demonstrate that loss of TDP-43 but not its mislocalisation augments paraspeckle assembly in cultured cells. TDP-43 is a component of the miRNA machinery, and recently, paraspeckles have been shown to regulate pri-miRNA processing raising the possibility that paraspeckle hyper-assembly in TDP-43-depleted cells is a mechanism to compensate for TDP-43 loss of function in miRNA biogenesis. Indeed, we show that downregulation of other key miRNA pathway factors also leads to enhanced paraspeckle assembly. In addition, loss of TDP-43 or miRNA pathway proteins results in abnormal accumulation of endogenous double-stranded (ds) RNA and activation of type I interferon response which also stimulates paraspeckle formation. Although human post-mitotic neurons lack paraspeckles in vitro, their de novo assembly can be triggered by a synthetic double-stranded RNA. We also provide evidence that paraspeckles protect cells from toxicity associated with depletion of TDP-43. Conclusions. Our study establishes possible mechanisms driving paraspeckle hyper-assembly in ALS and suggests their utility as therapeutic targets. Acknowledgments. We acknowledge London Neurodegenerative Diseases Brain Bank and Sheffield Brain Bank for providing human materials. The work was supported by a fellowship from Medical Research Foundation to TAS and a project grant Motor Neuron Disease Association (Buchman/Apr13/6096) to VLB.

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A17 Physical interaction and functional interplay of p62 and TDP-43 in ALS

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ALS is a complex, multifactorial disorder with diverse genetic and environmental components. Pathologically ALS is typified by the accumulation of proteinaceous cytoplasmic aggregates composed of abnormally ubiquitylated, hyperphosphorylated C-terminal fragments of the DNA/RNA binding protein TDP-43. Defects in proteostasis have been the subject of extensive investigation with regard to ALS, with various genes involved in the regulation of protein degradation including OPTN, TBK1, VCP, UQLN2, C9orf72 and SQSTM1 linked to familial forms. We previously provided evidence for functional defects in autophagy associated with disease-linked variants of the p62 protein (encoded by SQSTM1), both in vitro and in motor neurone-like cells [Goode et al. 2016 Autophagy]. However, the relationship between p62 dysfunction and ALS-relevant endpoints such as impaired TDP-43 metabolism remains incompletely explored. Here we present biochemical, biophysical and structural analyses that confirm a direct interaction between p62 and the canonical, pathogenic TDP25 fragment (residues 208-414). A defined binding site identified within p62 mediates the specific recognition of TDP25 with high (micromolar) affinity. The functional significance of this physical link between two ALS gene products, and the impact of ALS-linked mutations of p62, is explored.

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A18 Investigating a role for C9orf72 at the synapse

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative disorders which are linked clinically, pathologically and genetically. A key feature in both of these diseases is synaptic dysfunction, either at the neuromuscular junction or in the frontal and temporal lobes. This leads to neuron loss and the subsequent onset of both motor and cognitive symptoms. The most common familial cause of ALS/FTD is a GGGGCC repeat expansion in the C9orf72 gene. However, it is unclear how this mutation leads to neurodegeneration. Evidence exists in favour of three possible mechanisms: protein toxicity by the formation of dipeptide repeat proteins, RNA toxicity and haploinsufficiency. We report a novel role for C9orf72 at the synapse and hypothesise that loss of its function may be relevant to the onset of synaptic dysfunction in ALS/FTD. In order to further understand the role of C9orf72 in the cell, a yeast-two-hybrid screen was performed which identified potential interactions between C9orf72 and a few key synaptic proteins. These interactions were subsequently confirmed by co-immunoprecipitation and proximity ligation assay. In line with this, C9orf72 was found to be enriched in synaptosomes obtained by subcellular fractionation of mouse brain lysate. Additionally, miRNA-based knockdown of C9orf72 was performed in hippocampal neuron cultures which identified dysregulation of the levels of synaptic proteins in C9orf72-deficient cells. In summary, we report the presence of C9orf72 at the synapse and have identified novel interactions between C9orf72 and key synaptic proteins, whose levels may be dysregulated upon loss of C9orf72.

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A19 The C9orf72 protein interacts with mitochondria and regulates mitochondrial quality control

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Mitochondria are vital for the production of energy in the form of ATP as well as for the regulation of many cellular metabolic pathways, including calcium buffering and apoptosis. The maintenance of mitochondrial health is a dynamic process involving mitochondrial fission, fusion, transport and the removal of dysfunctional mitochondria by mitophagy. Due to their high metabolic requirements and extreme polarisation, neurons are especially dependent on the correct localisation and function of their mitochondria. Accordingly, they are selectively vulnerable to disturbance of mitochondrial quality control pathways. Mitochondrial dysfunction is strongly implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). A GGGGCC hexanucleotide repeat expansion in the first intron of the C9orf72 gene is the most common genetic defect associated with both ALS and FTD. It is unclear how the repeat expansion leads to disease, but both gain- and loss-of-function mechanisms have been proposed to contribute. As haploinsufficiency may play a role in C9orf72 ALS/FTD, identifying the function of the C9orf72 protein may inform on disease pathways. To investigate the function of the C9orf72 protein, a yeast two-hybrid (Y2H) screen was carried out to identify interacting partners of C9orf72. The Y2H screen identified several inner mitochondrial membrane (IMM) proteins as interactors of C9orf72, which were validated using co-immunoprecipitation. As expected of a protein that interacts with IMM protein complexes, a proportion of C9orf72 was found to localise to the mitochondrial intermembrane space. In order to elucidate the role of C9orf72 in mitochondria, we investigated its ability to regulate mitochondrial dynamics and mitophagy. Overexpression of C9orf72 led to fragmentation of the mitochondrial network. Conversely, siRNA knockdown of C9orf72 resulted in an increase in mitochondrial network connectivity and impaired clearance of damaged mitochondria by mitophagy. Taken together, our results show that C9orf72 localises to mitochondria and suggest that it is involved in mitochondrial quality control.

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A20 Study of mitochondrial function and mitochondrial fusion/fission dynamics in the cellular model of amyotrophic lateral sclerosis SOD1G93A NSC-34

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a selective loss of upper and lower motor neurons, culminating in a respiratory insufficiency and death 3-5 years after the beginning of the symptoms. ALS is the most common motor neuron pathology in the adult age, with an incidence rate of 2 per 100.000 persons-years. Most of ALS cases are sporadic, but approximately 10% of the patients show family inheritance, usually in dominant fashion. From the first description of a related gene with the disease, which encodes Cu/Zn superoxide dismutase protein (SOD1), many others have been described: TARDBP, FUS, C9ORF72, OPTN, ALS1, ALS2, UBQLN2, VCP, etc. Since then, many cellular and animal models have been developed showing that motor neuron degeneration appears multifactorial, and there is a confluence of pathogenic mechanisms that lead to cell death: mitochondrial dysfunction, oxidative stress, misfolded protein toxicity, autophagy defects, glutamate-mediated excitotoxicity, RNA toxicity and defective axonal transport. Moreover, several studies have evinced a defective energy metabolism in patients with ALS throughout disease progression. Increased energy expenditure (hypermetabolism) has been observed in both, sporadic and familial forms of the illness. In addition, whereas hyperlipidaemia is positively correlated with ALS survival, it doesn't happen with glucose, and there is a discussion about if type 2 diabetes is a detrimental factor or delays the disease onset. The aim of this work was to study mitochondrial function, assessed through the mitochondrial membrane potential measurement, to analyze reactive oxygen species production, to quantify total ATP levels and to evaluate mitochondrial network through the study of the fusion and fission mitochondrial proteins, when NSC-34 cells, expressing human SOD1G93A protein, are grown in normoglycemic and hyperglycemic conditions. This work was supported by Spanish Fundation for the research in amyotrophic lateral sclerosis (FUNDELA).

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A21 Identification and characterization of RANT modulators in the G4C2 expansion

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The intronic hexanucleotide repeat expansion, GGGGCC (or G4C2), in the C9ORF72 gene is the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Above 30 and up to hundreds repeats, G4C2 expansion is transcribed in aberrant mRNAs that fold in G-quadruplexes structures and generate RNA foci within motorneurons. Additionally, they also located in the cytoplasm and can be translated via the unconventional repeat-associated non-ATG translation (RANT) into five toxic dipeptide repeat (DPR) proteins that accumulate in cells. Unlike ATG-initiated translation, RANT products accumulate after activation of the integrated stress response (ISR). Both RAN foci and RANT products are considered the causative factors of the disease but, so far, no effective pharmacological approach is currently available. We performed a high-throughput drug-screening to identify modulators of RANT. Effective small molecules were divided into four categories: positive CAP and RANT products modulators, negative CAP and RANT products modulators, positive RANT products and negative CAP products modulators, negative RANT products and positive CAP products modulators. Selective hits were tested to evaluate their toxicity in a dose-response analysis and to assess whether their mechanisms of action affect general transcription and translation. Moreover, we verified whether they interfere with the ISR pathway by modulating the phosphorylation levels of PERK and eIF2Ð.

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A22 Glycosphingolipid dysregulation and lysosomal dysfunction in motor neurone disease

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Historically, the early-onset rare neurodegenerative lysosomal storage disorders (LSDs) have been studied as discrete metabolic diseases in their own right. However, recently some links with common late-onset neurodegenerative diseases, including motor neurone disease (MND), have emerged (Dodge, 2017; Tracey et al., 2018). Recent reports show lipid dysregulation, including increased levels of various glycosphingolipids (GSLs) and upregulation of enzymes responsible for GSL hydrolysis, in the spinal cord of MND patients and the mouse model SOD1(G93A) (Dodge et al., 2015). Inhibition of glucosylceramide (GlcCer) synthesis, the precursor of the vast majority of GSLs, accelerated disease progression and decreased lifespan, whereas infusion with GM3 ganglioside significantly delayed disease progression and increased survival, in the same mouse model (Dodge et al., 2015). Previous work in our laboratory found that GlcCer and downstream metabolism is significantly altered in spinal cord and muscle of SOD1(G86R) mice (Henriques et al., 2015), and inhibition of GlcCer catabolism preserved motor function and delayed disease onset (Henriques et al., 2017). Together, these studies highlight the importance of GlcCer and other GSLs for maintenance and regeneration of motor units. However, the relationship between the basic biochemical mechanisms linking lipid dysregulation, lysosomal dysfunction and altered lysosomal enzymatic activity in MND remains poorly understood. In this project, we are investigating the basic biological processes which cause lipid changes and lysosomal dysfunction in MND. We have started by characterising general changes in patterns of GSL expression in samples from SOD1(G86R), SOD1(G93A) and TDP-43(M337V) mouse models of MND. In agreement with previous reports, in spinal cord and soleus muscle of SOD1(G86R) mice (Henriques et al., 2015), total GSL levels in gastrocnemius and tibialis anterior of SOD1(G93A) mice increased with disease progression. On the other hand, the GSL content in spinal cord and tibialis anterior of SOD1(G86R) and in spinal cord and brain of TDP-43(M337V) did not change at age of onset of disease symptoms. Further studies are needed to determine the degree to which specific GSL changes are a common feature of MND, before, as well as after, the onset of overt symptoms.

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A23 C9ORF72 mutation impairs vesicular trafficking cell communication in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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† Both authors contributed equally to this work.

A non-coding hexanucleotide repeat expansion (HRE) in intron 1 of the C9ORF72 gene is the most common cause of amyotrophic lateral sclerosis and frontotemporal dementia (C9ALS/FTD), however, the precise molecular mechanism by which the C9ORF72 HRE directs C9ALS/FTD pathogenesis remains unclear. Here, we report a novel disease mechanism arising due to the interaction of C9ORF72 with the RAB7L1 GTPase. The C9ORF72 HRE resulted in severely defective intracellular and extracellular vesicle trafficking and a dysfunctional trans-Golgi network phenotype in patient-derived fibroblasts and iPSC-derived motor neurons. Genetic ablation of RAB7L1 or C9ORF72 in SH-SY5Y cells recapitulated the findings in C9ALS/FTD fibroblasts and iPSC-neurons. When C9ORF72 was overexpressed or antisense oligonucleotides were targeted to the C9ORF72 HRE to upregulate normal variant 2 transcript levels, the defective vesicle trafficking and dysfunctional trans-Golgi network phenotypes were reversed, suggesting that both loss- and gain-of-function mechanisms play a role in disease pathogenesis. In conclusion, we have identified a novel mechanism for C9ALS/FTD pathogenesis highlighting the molecular regulation of intracellular and extracellular vesicle trafficking as an important pathway in C9ALS/FTD pathogenesis. Further research will unravel the consequences of this impairment for neuromuscular junction and other tissues affected in ALS including skeletal muscle.

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A24 microRNAs analysis of patient-derived iPSCs and motor neurons for the development of a molecular therapy for ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of motor neurons (MNs). The pathomechanism underlying the disease is largely unknown, even though increasing evidence suggests that RNA metabolism, including microRNAs (miRNAs) may play an important role. Indeed, the occurrence of mutations in genes encoding for DNA/RNA-binding proteins, such as TDP-43 and FUS, and the hexanucleotide intronic repeat expansions in C9ORF72 supports this hypothesis. MiRNA are tissue-specific small molecules that can individually regulate several hundred targets by RNA-dependent mechanism. Since miRNAs are required for the survival of specific types of mature neurons in model organisms, they may play important roles in the aetiology or progression of neurodegenerative disorders. We and other groups have demonstrated that ALS-linked genes can affect miRNA expression. Here, we aim to investigate the role of miRNAs dysregulation and their relative proteomic changes in induced pluripotent stem cells (iPSCs) and motor neurons derived from ALS patients, based on in vitro models developed in our lab. We performed miRNA expression profiles analysis on iPSCs and their differentiated motor neurons in order to identify dysregulated miRNAs in ALS and we further characterized them and their biological targets by bioinformatic tools, molecular and proteomic studies. Taken together our results demonstrate that the neurodegenerative phenotype in ALS can be associated with a dysregulation of miRNAs involved in the control of disease-relevant genetic pathways, suggesting that targeting entire gene networks can be a potential strategy to treat complex diseases such as ALS.

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A25 Prognostic value of serum creatinine in ALS patients: A meta-analysis

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Creatinine is commonly measured in clinical practice to evaluate glomerular filtration rate or to indicate the severity of muscular atrophy. Serum creatinine may also be considered as a survival predictor for ALS patients. Several biomarkers were identified for ALS, but their clinical relevance in evaluating disease progression is a matter of debate. As the methodology of clinical trials is evolving towards the inclusion of new outcome biomarkers, the reliability of creatinine as a useful prognostic parameter is of utmost importance. Here, we conducted a meta-analysis to investigate the prognostic value of creatinine levels in ALS patients. We retrieved 13182 studies (68% from Medline, 27% from Embase and 5% from Cochrane Library) corresponding to scientific journal articles and abstracts from congress or specialized conferences. After a systematic review, we identified 20 studies with 15 distinct cohorts linking creatininemia and ALS progression (mortality/survival, ALSFRS-R score variation and at baseline). Different meta-analysis were performed because of the variety of disease progression parameters used in these studies. Two meta-analysis found a negative correlation between mortality and creatininemia at baseline used as a continuous variable (HR: 0.72; 95% confidence interval (CI): 0.58 – 0.88; $p = 0.0003$). A positive correlation was found between creatinine levels and functional score at baseline (0.3530; 95% CI: 0.2857 – 0.4168; $p < 0.0001$) or with variation in ALSFRS-R score (0.5857; 95% CI: 0.0674 – 0.8559; $p = 0.0146$). We also identified a positive correlation between the variation of creatinine levels and variation of functional score (0.377; 95% CI: 0.3321 – 0.4201; $p < 0.0001$). We found an elevated bias risk in the majority of studies analyzed. Most studies presented a high risk of bias of study attrition (80%; $n = 16$), as they are retrospectives, and few provided information on the number of missing data and number of patients lost in the follow-up during the study. Creatinine levels seems to be a promising indicator of ALS prognostics, but we suggest that novel studies must be conducted following consistent methodologies and standardized parameters for the evaluation of ALS progression in order to confirm the importance of creatinine levels as a valid clinical biomarker in ALS. Keywords: ALS, biomarkers, creatinine, meta-analysis.

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A26 Distinctive subcortical grey matter signatures along the ALS-FTD spectrum: a multimodal neuroimaging study

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Background: Phenotypes along the ALS-FTD spectrum have distinctive and well-established cortical signatures, but their subcortical grey matter profiles are poorly characterised. The comprehensive characterisation of striatal and thalamic pathology along the ALS-FTD spectrum is particularly timely, as dysfunction of frontostriatal and cortico-thalamic networks contribute to phenotype-defining cognitive, behavioural, and motor deficits. Methods: Fourteen ALS-FTD patients with C9orf72 hexanucleotide expansions, 12 ALS-FTD patients without hexanucleotide repeats, 36 ALS patients without cognitive impairment, 10 patients with behavioural-variant FTD, 11 patients with non-fluent-variant primary progressive aphasia, 5 patients with semantic-variant primary progressive aphasia and 50 healthy controls were included in a prospective neuroimaging study. Striatal, thalamic, hippocampal and amygdala pathology was evaluated using volume measurements, density analyses and connectivity-based segmentation. Results: C9-positive ALS-FTD patients showed preferential density reductions in thalamic sub-regions connected to motor and sensory cortical areas. C9-negative ALS-FTD patients exhibited striatal pathology in sub-regions projecting to rostral-motor and executive cortical areas. Significant volume reductions were identified in the thalamus and putamen of non-fluent-variant PPA patients. Marked nucleus accumbens and hippocampal atrophy was observed in the behavioural-variant FTD cohort. Semantic-variant PPA patients only exhibited volumetric changes in the left hippocampus. The bulk of striatal and thalamic pathology in non-fluent-variant PPA patients was identified in foci projecting to motor areas. Subcortical density alterations in svPPA patients were limited to basal ganglia regions with parietal projections. Conclusions: Striatal and thalamic changes in FTD exhibit selective, network-defined vulnerability patterns mirroring cortical pathology. Multi-modal cortico-basal imaging analyses confirm that the subcortical grey matter profiles of FTD phenotypes are just as distinct as their cortical signatures. Our findings support emerging concepts of network-wise degeneration, preferential circuit vulnerability and disease propagation along connectivity patterns.

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A27 The width of the third ventricle in ALS patients reflects subcortical gray matter atrophy and associates to cognitive impair

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Background An enlarged width of the third ventricle (WTV) has been described in amyotrophic lateral sclerosis (ALS) patients, although their meaning is unknown. The aim of this study was to evaluate the contribution of demographical, clinical and genetic factors to the WTV in different ALS phenotypes and to assess its brain structural correlates. Methods The WTV was measured by means of transcranial ultrasound in 107 patients, diagnosed with classical ALS (82), progressive muscular atrophy (16) and primary lateral sclerosis (9), and 25 controls. In 85 ALS patients brain volumetric analysis of MR images were additionally implemented. The association of the WTV with demographic, clinical, genetic and neuropsychological variables as well as with brain volumes was assessed with multivariable models. Results Eighteen patients were diagnosed with genetic ALS and 42.3% of patients showed executive or behavioural impairment (EBI). ALS patients showed larger WTV than controls. The WTV associated with demographical (age and male sex) and EBI (Estimate = 0.229 [0.024, 0.434], p = 0.029), but not with the genetic background, the motor phenotype or disability. Greater WTV associated with reduced total subcortical gray volume (Estimate = -0.211 [-0.393, -0.03], p = 0.023), but not with the cortex volume or the cortical white matter volume. Conclusions The enlargement of the WTV found in the different ALS phenotypes is attributable to the atrophy of subcortical gray matter structures and associates to cognitive and behavioural impairment. Larger longitudinal studies are needed to determine its role as a diagnostic or prognostic biomarker, especially in ALS-FTD patients.

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A28 Motor unit number index (MUNIX) in proximal muscles of the arm in amyotrophic lateral sclerosis

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Introduction: MUNIX is an easy-to-perform and well tolerated electrophysiological technique for quantitatively assessing the loss of lower motor neurons. Previous studies have shown that MUNIX could be a relevant method to monitor disease progression in amyotrophic lateral sclerosis (ALS). Most studies have measured MUNIX in abductor digiti minimi (ADM), abductor pollicis brevis (APB) or tibialis anterior. However, distal muscles are often rapidly atrophied in ALS, which makes MUNIX no more measurable. Hence, it would be interesting during longitudinal follow-up of ALS patients to study proximal muscles which are often later affected. Yet nerve conduction study of proximal muscles is technically more complicated. The aim of this study was to test the feasibility of MUNIX in proximal arm muscles in ALS patients. Methods: 31 ALS patients (24 spinal and 7 bulbar onset ALS) and 16 healthy subjects were prospectively included. MUNIX, compound muscle action potential (CMAP) and motor unit size index (MUSIX) were studied in biceps, triceps, deltoid, ADM and APB muscles. Results: MUNIX and CMAP were significantly decreased in each muscle in ALS patients compared to healthy subjects ($p < 0.01$). MUSIX was increased in ADM, APB and biceps in ALS patients compared to healthy subjects ($p < 0.01$). MUNIX sum score (ADM + APB + biceps + triceps + deltoid) and MUNIX sum score of the proximal muscles (biceps + triceps + deltoid) correlated with ALSFRS-R ($rho = 0.5$, $p < 0.01$). MUNIX correlated with the MRC muscle scale of APB, biceps and deltoid ($rho = 0.7$, $p < 0.01$). Conclusion: This study shows that MUNIX of proximal arm muscles is a feasible technique and correlates with clinical disability in ALS, and therefore could be used in the global MUNIX assessment of ALS patients as biomarker of the disease.

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A29 The clinical and radiological landscape of PLS: A multimodal neuroimaging study

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Background: Primary lateral sclerosis (PLS) is a progressive upper motor neuron disorder which is often seen as an UMN-dominant phenotype on the ALS spectrum. The distinguishing radiological features of PLS compared to ALS are relatively poorly characterised. Objectives and Methods: Our study aims to comprehensively evaluate a large cohort of PLS patients using clinical, neuropsychological and multiparametric MRI. Results: 105 participants (19 PLS patients, 50 healthy controls and 36 ALS patients) were included in a prospective neuroimaging study with standardised clinical assessments. In the PLS cohort, the mean age at diagnosis is 55.2 years, mean symptom duration is 10.7 years. Lower limb onset is reported by all 19 subjects with subsequent upper limb Eoin Finegan*(1), Rangariroyashe H. Chipika (1), Orla Hardiman (1), Peter Bede (1) (n=14, 74%). The disability profile of PLS patients also suggests an ascending pattern with symmetric limb spasticity, greater in the lower compared with upper limbs and relative sparing of bulbar functions(Mean ALSFRS subscores; gross motor 5.67, fine motor 8.33 and bulbar 9.42. Mean Penn UMN subscores; lower limbs 9.67 max=14, upper limbs 6.89 max=14 and bulbar 1.22 max=4). There is a significant inverse correlation between tapping rates and both the Penn UMN and spasticity scores. Precise assessment of tapping rates seem to be a useful indicator of UMN disease burden in PLS. Pathological crying and laughing (PCL) is highly prevalent in our cohort and is associated with significant distress. Using the Emotional Lability Questionnaire, 45% of the PLS group had severe symptoms. PCL is strongly correlated with the bulbar subscore of the ALSFRS (r= -0.817, p= 0.002), although not with total ALSFRS (r= -0.293, p= 0.382). Our imaging data suggest that PLS patients have marked precentral gyrus atrophy. Consistent with the clinical profile of PLS, the bulbar segment of the motor cortex is relatively spared. Extra-motor involvement is limited to a small left mesial temporal focus. White matter analyses identified bilateral corticospinal tract degeneration highlighted by decreased fractional anisotropy, increased radial and axial diffusivity. White matter degeneration is limited to the posterior limb of the internal capsule with relative sparing of the corticobulbar fibres in the genu. Conclusions: Multiparametric imaging of clinically well characterised PLS patients confirm distinguishing pathological signatures from ALS.

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A30 CSF and serum pNfH assay performance study in the ALS clinic

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Background Multiple studies spanning more than 20 years have demonstrated increased neurofilament levels in the serum and CSF of patients with ALS and healthy controls. These studies have increasingly extended to the differentiation of ALS from mimic disorders and to consider NFs as a prognostic biomarker and its potential marker of therapeutic response. Methods We tested the performance of a CE-marked ELISA (Euroimmun, Germany) for the measurement of pNfH in matched serum and CSF from the â€˜BioMOxâ€™ longitudinal cohort comprising ALS patients (CSF = 75, serum = 72), healthy controls (CSF = 28, serum = 27), disease mimics seen in a tertiary ALS referral centre (n=12), and asymptomatic gene carriers of ALS-causing dominant gene mutations (n=3). We assessed first-visit pNfH in relation to absolute ALSFRS-R, its rate of change, and in relation to survival. Six-monthly longitudinal sample levels in the ALS group were also measured. A linear mixed model of CSF and serum was fitted to longitudinal values; beginning at the baseline visit, with patients stratified by rate of decline of ALSFRS-R at baseline visit in relation to median value. Results As expected, pNfH levels were higher in both ALS patients versus healthy controls (CSF $p<0.0001$; serum $p<0.0001$) and versus disease mimics (CSF $p<0.0001$; serum $p = 0.0099$). CSF and serum pNfH levels were not significantly raised in asymptomatic gene carriers versus healthy controls. Using ROC analysis, optimal pNfH cut-off discriminated patients with ALS from healthy controls (CSF AUC 0.98; serum AUC 0.82). pNFH retrospectively differentiated patients with ALS from disease mimics (CSF AUC 0.92; serum AUC 0.80). CSF and serum pNfH concentrations correlated in patients with ALS ($r=0.46$, $p<0.0001$). Correlations were considered between pNfH levels and rate of decline of ALSFRS-R (CSF $r=0.69$, $p<0.0001$; serum $r=0.30$, $p=0.1$ NS), and absolute ALSFRS-R (CSF $r=-0.16$, $p=0.18$; serum $r=0.37$, $p<0.005$). Survival analysis showed separation of groups of patients binarized into high and low first-visit pNfH levels (CSF $p<0.001$, serum $p<0.05$). CSF and serum pNfH levels did not significantly change over time. Conclusion A commercially available pNfH assay validated for clinical diagnostics has meaningful diagnostic accuracy for ALS in a tertiary referral clinic setting. CSF and serum pNfH levels also provide prognostic information and have very clear further potential as markers of future therapeutic effect.

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A31 Circulating exosomes as a promising source of biomarkers for ALS

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Reliable and truly validated biomarkers of disease progression in amyotrophic lateral sclerosis (ALS) are not available yet. Identifying biomarkers enabling an objective disease monitoring would speed up development of ALS treatments. Extracellular vesicles (EVs) are membrane surrounded structures released by cells and circulating in biological fluids. EVs are involved in intercellular communication, carrying a variety of cargos such as RNA, metabolites and proteins, including those prone to aggregation (e.g. SOD1 and TDP-43). We and others have shown that normal cells release EVs constitutively, but increase their release in response to a variety of pathological conditions, including ALS, probably contributing to disease spreading and progression. Plasma concentration of EVs and their biochemical properties are therefore accessible and measurable parameters that may underline disease progression and differentiate ALS from other pathological conditions. In this study we tested the feasibility to use circulating exosomes as a source of biomarkers for ALS. We first took advantage of a newly established protocol to purify EVs that preserve their morphology and physical and biochemical properties. Next, we analyzed concentration and size of EVs from plasma of ALS patients and controls (healthy, neurological and muscular dystrophy) by tunable resistive pulse sensing-based technology. We found that ALS EVs are smaller and more abundant and their number increases with the progression of the disease. Furthermore, patients with slower progression of the disease have an higher number of EVs in plasma. Finally, we analyzed the level of a priori-selected candidate biomarkers (TDP-43, cyclophilin A, heterogeneous nuclear ribonucleoprotein A2/B1 and SOD1) and demonstrated that circulating exosomes are a promising source of biomarkers that can be used in large clinical studies.

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A32 The TDP-43 pathological interactome

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Background: Identification of biomarkers is needed to facilitate effective therapy development in ALS. TDP-43 pathology is a characteristic feature of nearly all cases of ALS, and half of those with the linked condition of frontotemporal dementia (FTD). TDP-43 is a heterogeneous nuclear ribonucleoprotein (hnRNP) including a C-terminal glycine-rich domain important for binding to homo- and heterotypic proteins. The study of proteins which interact with pathological forms of TDP-43 might advance biomarker development and illuminate key pathways for therapeutic targeting. Objective: To identify the pathological TDP-43 interactome in a cellular model of ALS. Method: Mouse embryonic stem cells used to make a novel (BAC)-transgenic mouse model carrying GFP-tagged human TDP-43 (M337V mutant and wildtype TDP-43) were expanded to embryoid bodies and then differentiated to motor neurons. Motor neuron cultures (non-stressed or stressed (0.5mM NaAr 1h) were co-immunoprecipitated using an anti-GFP antibody, followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify human TDP-43 and its binding partners. Results: Co-immunoprecipitation of GFP-tagged TDP-43 led to highly specific and efficient pull-down of human TDP-43 shown by a 72kDa band in western blots. Following LC-MS/MS analysis of the co-immunoprecipitated eluate, human TDP-43 interacting proteins were identified. Statistical analysis revealed interacting proteins with differential binding to wildtype and M337V mutant TDP-43. Conclusion: Identification of the disease-relevant TDP-43 interactome in this model will enable subsequent validation of TDP-43 interacting proteins in human induced pluripotent stem cells, human post mortem tissue and biofluids, towards the goal of disease-specific biomarker development in ALS.

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A33 Connectivity-based thalamic segmentation as a cortical pathological window in ALS

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Background The ALS-FTD syndrome involves a widespread but systematized cortical pathology. The few histopathological studies in ALS reporting degeneration in the thalamus and associated nerve fibres have been corroborated by modern MRI studies. PET also revealed reduced thalamic task-related activation, hypometabolism, and marked microglial activation. Assessment of intra-thalamic features in relation to cortical connectivity offers a window on the extent of cerebral ALS pathology, and post mortem MRI allows direct comparison with histological changes. Objectives To attempt connectivity-based thalamic segmentation and examine regional variation using post mortem MRI. Methods Ultra-high field 7T MRI was undertaken in donated brains from 5 ALS patients (age at death = 68 ± 9 years) and 3 controls (age at death = 65 ± 8 years) currently undergoing histopathological sampling. Atlas 'target' masks of frontal, temporal, parietal, occipital, and motor cortices were registered to each brain's DTI space. Thalami were parcellated into sub-regions based on their level of connectivity with the cortical targets using probabilistic tractography. Tractography results were subjected to 1) hard segmentation (thalamic voxels were assigned to the target mask with which they showed highest connectivity), and 2) parcellation based on thresholding thalamus-to-target connectivity outputs at 25% of their maximum value. All resulting volumes, DTI metrics extracted from parcellations obtained via approach 2, and results for the overall thalami were normalised by the respective values for the occipital lobe related region to correct for inter-subject variability introduced by age, post mortem delay and fixation time. Group comparisons were performed using permutation tests (PALM). Results Significantly smaller volumes were present in ALS for left thalamic regions chiefly connected to the primary motor cortex and temporal lobe, as well as for a right sub-region primarily connected to the parietal lobe. DTI-FA was significantly reduced in ALS averaged across the whole left thalamus, and more specifically in sub-regions defined by their connectivity with BA6 and the parietal lobe. Conclusion ALS pathology may involve changes in motor and pre-motor cortical, as well as temporal and parietal lobe thalamic representations. Comparison with histological data will shed light on the accuracy of tractography-based thalamic segmentation, and its value for patient stratification.

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A34 Oxidation-reduction potential of cerebrospinal fluid as progression biomarker in ALS patients with spinal onset

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Oxidative stress has been a subject of research and a valid therapeutic target for ALS treatment since the discovery of SOD1 mutations in familial cases. Oxidative stress-related pathological mechanisms (may) involve cell death-related release of pro-oxidative compounds and redox-active iron, mitochondrial dysfunction, inflammation, and excitotoxicity. This can be exploited in the development of ALS biomarkers. The lack of reliable biomarkers represents a bottleneck in clinical trials and the development of novel drugs. The properties of cerebrospinal fluid (CSF) reflect the processes in the spinal cord and brain tissues, making CSF a good vehicle for developing biomarkers of neurodegeneration. We hypothesize that multiple oxidative pathways and complex biology of ALS can be captured by an integrative parameter, such as oxidation-reduction potential (ORP), a comprehensive metabolic analyte that measures the balance between oxidative and reductive species in biological fluids. ORP was measured using RedoxSYS (Aytu BioScience, Inc., Englewood, CO, USA) in CSF of 82 ALS patients and 24 age-matching controls. ORP was significantly higher in ALS patients compared to controls. Receiver operating characteristic curve's area under curve (AUC) for both all (AUC = 0.665, P = 0.014) and spinal onset patients (SOP) (AUC = 0.690, P = 0.006) was significant. The optimal cut-off was at 108.4 mV (72% sensitivity, 58% specificity). This ORP value can be considered as threshold that define a point at which redox status of CSF is affected by pro-oxidative events in CNS of ALS patients. ORP correlated significantly with ALSFRS-R score for all, SOP and male patients (R = -0.369, R = -0.464 and R = -0.488, respectively; P < 0.001) and even stronger for above-the-threshold subgroups (R = -0.423, R = -0.573 and R = -0.593, respectively; P<0.001). In SOP group, the survival time from sample collection to death correlated significantly with ORP for ORP values over 125 mV (R = -0.600, P = 0.023) implying that high ORP correlates with shorter survival time. Increased ORP values in patients further confirms the role of oxidative stress in pathology of ALS. ORP represents a viable candidate for the biomarker of progression and/or prognosis, as it fulfils FNIH criteria for biomarker development. It is an objective parameter (range 90-190 mV, increment 0.1 mV), the assay is simple, cheap, and can be performed at the bedside.

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A35 Implementing Motor Unit Number Index (MUNIX) in a large clinical trial: Real world experience from 27 centres

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Objective: Motor Unit Number Index (MUNIX) is a quantitative neurophysiological method that reflects loss of motor neurons in Amyotrophic Lateral Sclerosis (ALS) in longitudinal studies. It has been utilized in one natural history ALS study and one drug trial (Biogen USA) after training and qualification of raters. Methods: Prior to testing patients, evaluators had to submit test-retest data of 4 healthy volunteers. Twenty-seven centers with 36 raters measured MUNIX in 4 sets of 6 different muscles twice. Coefficient of variation of all measurements had to be <20% to pass the qualification process. MUNIX COV of the first attempt, number of repeated measurements and muscle specific COV were evaluated. Results: COV varied considerably between raters. Mean COV of all raters at the first measurements was 12.9% ± 13.5 (median 8.7%). Need of repetitions ranged from 0 to 43 (mean 10.7 ± 9.1, median 8). Biceps and first dorsal interosseus muscles showed highest repetition rates. MUNIX variability correlated considerably with variability of compound muscle action potential. Conclusion: MUNIX revealed generally good reliability, but was rater dependent and ongoing support for raters was needed. Significance: MUNIX can be implemented in large clinical trials as an outcome measure after training and a qualification process.

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A36 Discovery and development of diagnostics and therapeutics for TDP-43 proteinopathies

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TDP-43 is multifunctional and essential RNA-binding protein (RBP), whose cytoplasmic aggregation is the molecular basis for neurodegeneration in the majority of patients with amyotrophic lateral sclerosis (ALS) and in about 45% of patients with frontotemporal dementia (FTD). Moreover, TDP-43 copathology is also found in sub-populations of Alzheimer's disease (AD), Huntingtons' disease (HD), Lewy body diseases, Pick's disease etc (1), but their contribution to disease is currently unknown. Neurodegenerative diseases linked to deposition of TDP-43 are therefore classified as TDP-43 proteinopathies. Even though the loss of normal nuclear localization and cytoplasmic TDP-43 aggregation correlates with neurodegeneration, the exact mechanisms of neurotoxicity remain elusive. Nonetheless, recent research suggests that similar to other protein aggregation diseases, TDP-43 proteinopathies follow the prion paradigm through templated conversion and spread of pathologic conformers across the central nervous system (CNS). Antibody-mediated clearance of pathological TDP-43 aggregates therefore represents an attractive strategy for therapeutic intervention. However, the lack of tools for accurate diagnosis and monitoring of disease progression have impeded the research and development of therapeutics for TDP-43 proteinopathies.
We have generated antibodies that specifically recognize pathological TDP-43 inclusions in post-mortem brain tissue from ALS and FTD patients. These antibodies display high affinity and selectivity for misfolded TDP-43 in vitro and are currently being evaluated for their therapeutic potential. To complement the therapeutic approach, we are generating small molecules suitable for further development as positron emission tomography (PET) ligands. We have identified a set of small molecules that specifically bind to pathological TDP-43 in post-mortem brain tissue and display suitable CNS PET properties. Additional compounds are currently being synthesized and evaluated with the goal to develop PET ligands with high affinity for TDP-43 while being selective over amyloid-beta and tau aggregates.
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A37 Cervical spinal cord comparisons based on T1-weighted MRI in ALS

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Most neuroimaging research in ALS has focused on imaging the brain revealing reduction of cortical thickness and white matter tract connectivity. On brain MRI scans, the upper part of the cervical spinal cord is also captured. As the spinal cord could reveal both upper and lower motor neuron (UMN and LMN) involvement, spinal cord neuroimaging may be capable of capturing characteristics of ALS. In this study, the cross-sectional area (CSA) of the upper part of the cervical spinal cord on brain MRI scans was investigated to detect disease effects. 302 subjects (108 sporadic ALS patients, 28 primary lateral sclerosis (PLS) patients, 58 progressive muscular atrophy (PMA) patients and 108 controls) were included of whom T1-weighted images were acquired. For each subject, the spinal cord was segmented and divided into the different cervical segments. For each slice of the segmented spinal cord the CSA was computed and the CSAs of all slices in the first three cervical segments (C1 - C3) were averaged to obtain one value for upper spinal cord thickness. Compared to controls, lower spinal cord CSA was observed in PMA ($p=0.017$, difference mean = -3.10, 95% CI [-5.86;-0.34]), ALS ($p=0.001$, difference mean= -3.54, 95% CI [-5.65;-1.44]) and PLS ($p<0.001$, difference mean= -8.71, 95% CI [-14.20;-3.22]). Patients with PLS showed the thinnest upper spinal cord compared to the other groups. These results demonstrate the ability of spinal cord MRI to capture disease effects, and indicate spinal cord MRI to be a modality to consider for neuroimaging in ALS.

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A38 Analysis of GAP-43 expression in differentially vulnerable muscles in two mouse models of motor neuron disease

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In amyotrophic lateral sclerosis (ALS) the axonal connection with the target muscle – the neuromuscular junction (NMJ) – is affected early in disease. However, while motor axons of fast-fatigable motor neurons start dying back, the more resilient slow motor neurons undergo a period of axonal sprouting and compensatory growth, as demonstrated in the mutant SOD1 mouse model of ALS. It is now yet known if this compensatory sprouting is a general feature of slow motor neurons across disease models. In addition to this intra-muscular difference in sprouting capacity there are differing levels of vulnerability to disease between motor neurons. In particular the motor neurons from the oculomotor nucleus which innervate the muscles surrounding the eye are spared in ALS, and these extraocular muscles remain functional late in the disease. It is not known if these motor neurons have the capacity to sprout or if their resilience across motor neuron disease is based on mechanisms distinct from those seen in slow motor neurons. Here we have compared the regenerative capacity of differentially vulnerable muscles in ALS using GAP43 as a marker of regeneration. We have utilised a range of muscles from two mouse models of motor neuron disease with differing patterns of muscle vulnerability and resistance – SOD1G93A and pmn (progressive motor neuronopathy) – to investigate muscle specific responses to different disease-causing mutations.

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A39 Mutations in FUS lead to axonal and synaptic changes in a zebrafish model and primary cortical neurons

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive fatal neurodegenerative diseases, which no effective therapies currently exist. In 2009, mutations in an RNA binding protein Fused in Sarcoma (FUS) were identified as causative to an aggressive form of ALS which present large pathological neuronal cytoplasmic FUS-positive aggregates and concomitant loss of FUS from the nucleus in neurons (Vance, 2009). The majority of mutations in the extreme C-terminus lead to this phenotype as the nuclear localising signal are thought to be disrupted. Aims: 1) To investigate how overexpression of mutant FUS affects axonal and synaptic growth in primary cortical cells. 2) Assess the effects of overexpressed mutant FUS in primary motor neurons in the zebrafish. Methods: 1) Human FUS was microinjected and transfected into the zebrafish and primary cells respectively. Effects were analysed at 2DPF in vivo and DIV8 in vitro via confocal microscopy. 2) Primary motor neuron changes were assessed by driving FUS expression by an MNX: Gal4 plasmid. Results: Preliminary data suggest that, in comparison with wildtype, expression of the FUS K510X mutant causes gross axonal and synaptic (pre/post) changes with overt cytoplasmic localisation, whereas the R514G mutant shows weak cytoplasmic expression, imposing modest changes to axonal and synaptic development in vitro and in vivo. Conclusion: We can conclude that K510X causes overt cytoplasmic expression causing axonal and synaptic degeneration whereas R514G leads to a milder phenotype. This could elucidate two separate mechanisms for these different mutations which should be investigated further.

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A40 Deciphering the dual neuroprotective/neurotoxic role of FGF-2 in SOD1G93A ALS mice in vitro and in vivo

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We have previously shown that knock out of fibroblast growth factor 2 (FGF-2) and potential compensatory effects of other growth factors result in amelioration of disease symptoms in a transgenic mouse model of amyotrophic lateral sclerosis (ALS). ALS is a rapidly progressive neurological disorder leading to degeneration of cortical, brain stem and spinal motor neurons followed by subsequent denervation and muscle wasting. Mutations in the superoxide dismutase one (SOD1) gene are responsible for about 20% of familial ALS cases and SOD1 mutant mice still are among the models best mimicking clinical and neuropathological characteristics of ALS. The aim of the present study was a thorough characterization of FGF-2 and other growth factors and signaling effectors in vivo in both SOD1G93A and double transgenic SOD1G93A-FGF-2 deficient mouse models. We observed tissue specific converse gene regulation of FGF-2 and overall dysregulation of other growth factors which in the gastrocnemius muscle was associated with reduced downstream extracellular signal-regulated kinases 1/2 (ERK1/2) and protein kinase B (AKT) activation. To further investigate whether the effects of FGF-2 on motor neuron death are mediated by glial cells, astrocytes lacking FGF-2 were co-cultured together with mutant SOD1G93A motor neurons. While FGF-2 knock out was not protective against staurosporine (STS) induced apoptosis, it had an impact on motor neuron maturation and synaptic activity indicating that astrocytic FGF-2 affects motor neurons at a developmental stage. Moreover, neuronal gene expression patterns showed FGF-2 and SOD1G93A dependent changes in growth factors and ERK2 implying a potential involvement in ALS pathogenesis before the onset of clinical symptoms.

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A41 Blocking Carnitine palmitoyl-transferase 1 (CPT1) potentially delays disease progression in the SOD1 G93A mouse model

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Background: Amyotrophic lateral sclerosis (ALS) has long been recognized as a neurodegenerative disease. However, several recent findings indicates that ALS should be understood as a multisystem disease. When comparing the diseases ALS, multiple sclerosis (MS) and depression, there are a number of common pathogenic/metabolic mechanisms. 1) all include an inflammatory component, 2) stress is a critical factor 3) lipid metabolism is upregulated, 4) humans with reduced lipid metabolism (inherited specific CPT1a gene mutation) are protected from MS and Depression, 5) Humans with MS or ALS have a relative high prevalence of depression. One key aspect in ALS, MS and depression is dysregulation of metabolism in general and alteration of lipid metabolism in particular. Blocking CPT1 in animal models of MS and depression has shown significant therapeutic effects. Therefore, the aim of this study was to examine the effect of blocking CPT1 in the SOD1 G93A animal model of ALS. Methods: Male B6.Cg-Tg(SOD1*G93A)1Gur/J mice were bought from Jackson Laboratories and mated with female C57Bl6/J. Female transgenic mice and their wild type littermates were weighed and assessed with a neurological score system weekly from 40 days of age (week 1) and assessed with a hangwire test from day 43. The mice were tested weekly with a rotarod apparatus from day 55 (week 3). When the animals reached day 100 (week 9) they were randomized into two groups. One group was treated with the CPT1-blocker, etomoxir, and the second group was treated with olive oil daily until they reached 16 weeks of age or on ethical grounds were terminated. Results: The transgenic mice (n=7) treated with the CPT1-blocker showed significant lower weight loss, improved neurological disease score and performed significant better at the rotarod- and the hangwire test compared to the placebo group (n=3) at several time points. Conclusion: The data obtained in this study indicates that blocking lipid metabolism through the blockade of CPT1 has the potential to be a highly effective treatment of ALS. However, further studies are required.

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A42 Inhibition of B-Glucocerebrosidase activity preserves motor unit integrity in a mouse model of amyotrophic lateral sclerosis

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Multiple lines of evidence suggest a link between sphingolipid metabolism and the physiopathology of amyotrophic lateral sclerosis [1]. Glucosylceramide, a sphingolipid, is the precursor of gangliosides. And degradation of glucosylceramide is performed by GBA1 and GBA2, two beta-glucocerebrosidases. Our previous results have shown a benefit for SOD1G86R mice after inhibition of glucosylceramide degradation [2]. Ambroxol hydrochloride is a safe and generic drug known to inhibit GBA2 activity. In SOD1G86R mice, an animal model of amyotrophic lateral sclerosis, ambroxol preserves neuromuscular junctions from denervation, delays disease onset, improves motor function and preserves motor neurons from degeneration. Taken together, our results suggest that GBA2 is a therapeutic target for ALS and that its inhibition preserves motor unit integrity in the SOD1G86R mice. In addition, our results suggest that ambroxol hydrochloride is a candidate drug for this devastating disease. [1] Henriques et al. 2017 Frontiers in Mol neuroscience [2] Henriques et al. 2017 Scientific reports

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A43 Study of the mechanisms leading to immune disorder in C9orf72 deficient mice

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An expansion of GGGGCC repeats in the first intron of the C9ORF72 gene is the most common genetic cause of Amyotrophic Lateral Sclerosis associated to Frontotemporal Dementia (ALS/FTD). As a decreased expression of C9ORF72 is observed in patients carrying expansions, we created a C9orf72 deficient mice to assess whether C9ORF72 haploinsufficiency could induce an ALS phenotype. However, those KO mice developed a splenomegaly and lymphadenopathy. Sera analysis and histopathology revealed an elevation of autoantibodies and a glomerulonephropathy, leading to a reduced mice longevity. In order to investigate further this phenotype, we crossed our C9orf72 flox mice with Cre lines specific of the main immune cell populations: dendritic cells (CD11c Cre), macrophages (LysM Cre), B cells (Mb1 Cre) and T cells (CD4 Cre). Interestingly, C9orf72 flox X CD11C CRE mice developed massive splenomegaly and lymphadenopathy. In contrast, deletion of C9orf72 in B cells, T cells or macrophages did not induce any phenotype. These results indicate a major role of C9orf72 in dendritic cells. Immunophenotyping of the DC lineage, from the myeloid precursor to conventional DCs and plasmacytoid DCs (pDC) of C9orf72 knockout mice demonstrated a specific alteration of the pDCs, which are more numerous and immature compared to control mice. Plasmacytoid dendritic cells are specialized immune cells dedicated to respond to viral infection by massive secretion of interferon. Currently, we are trying to reproduce those results in cell culture using bone marrow derived pDCs, and measure their response and interferon secretion upon stimulation mimicking viral infection. Overall, these results suggest a crucial function of C9ORF72 in dendritic cells. Hopefully, this study will help to better assess the molecular and cellular functions of C9ORF72 and to better understand its implication in amyotrophic lateral sclerosis.

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A44 The oculomotor-restricted protein Synaptotagmin 13 protects motor neurons from degeneration in ALS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective and progressive death of somatic motor neurons (MNs). However, not all somatic MNs degenerate in ALS; certain groups of MNs, including those in the oculomotor nucleus (OMN), appear resistant to degeneration. Mechanisms responsible for MN subtype-selective resistance and vulnerability in ALS remain largely unknown. Elucidating the molecular basis for the selective resistance of OMNs may lead to development of new therapies to prevent the relentless MN loss in ALS. We demonstrate that OMNs show preferential expression of synaptotagmin 13 (SYT13) which is maintained in OMNs and remaining spinal MNs in end-stage ALS patient tissues, supporting a role in their relative resistance. Overexpression of SYT13 in human ALS in vitro models improves MN survival and increases motor axon length. Adeno-associated virus-mediated delivery of Syt13 to transgenic ALS mouse model reduces pathology, delays muscle denervation and prolongs survival. The mechanisms underlying the induced neuroprotection are multifactorial including the reduction of endoplasmatic reticulum stress and apoptosis. Overall, our study suggests that elucidating mechanisms of differential neuronal vulnerability may lead to the identification of new therapeutic strategies to prevent the progressive degeneration in ALS.

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A45 Newly established ALS model of long-living double mutant hSOD1/RAG2/-/- mice could be attractive for testing therapeutic utility of human stem cells

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease with no effective cure that's why stem cell-based approaches are of particular interest with the potential to provide novel therapy. However, in order to test the efficacy of stem cells in animal models, there is a need to apply appropriate model for cell transplantation. One of the basic problems in human stem cell replacement strategies is the time needed for cells to become functional. The lifespan of most popular high copy number SOD1 mice (ALS model) might be too short (about 130 days) to realize full advantages of transplanted cells. Therefore, we focused on developing immunodeficient rag2-/- model of ALS that would have lower copy of transgene and thus longer lifespan. Methods: Our SOD1/rag2-/- double mutants were characterized genetically (qPCR). Anatomical changes in the brain were performed with the use of magnetic resonance imaging (MRI). The degree of motor neuron degeneration in the brain of SOD1/rag2-/- mice was evaluated by staining with Fluoro-Jade C®. Additionally the accumulation of misfolded SOD1 protein was estimated. The analysis of morphology and the number of motor neurons in the spinal cord was performed using cresyl violet staining. Results: The obtained SOD1/rag2/-/- mice colony has been characterized by qPCR analysis of a copy number of hSOD1 gene. qPCR performed against the standards revealed that progeny in our colony had either 4 or 8 copies of hSOD1 gene. The characteristic features of this ALS model like phenotype, protein accumulation and motoneurons degeneration are still preserved despite the low copy number of transgene. The difference in transgene copy number have been translated to significant impact on the animal life-span (157 days in 8-copy mice and 256 days in 4-copy mice). The death of both hSOD1/rag2 mice was preceded by muscular weakness as early as one month before death. Importantly, we were able to see the difference in signal intensity in T2-weighted MRI in medulla in terminal stage of the disease. Misfolded SOD1 accumulation was visible in cortical neurons and in spinal cord already in pre-symptomatic stage of disease. Conclusions: We developed long-living double mutant hSOD1/rag2/-/- mice, which could be very attractive for testing therapeutic utility of human stem cells. Our preliminary studies using this model for hGRP transplantation are in progress. Supported by NCR&D grants: STRATEGMED GRP&ALS & ERA-NET EuroNanoMed II "NanoTech4ALS"

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A46 MIF inhibits the formation and toxicity of misfolded SOD1 amyloid aggregates: Implications for familial ALS

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Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease caused by the progressive loss of motor neurons in the brain and spinal cord. It has been suggested that toxicity of mutant SOD1 results from its misfolding, however, it is yet unclear why misfolded SOD1 accumulates specifically within motor neurons. We recently demonstrated that macrophage migration inhibitory factor (MIF)-a multifunctional protein with cytokine/chemokine activity and cytosolic chaperone-like properties-inhibits the accumulation of misfolded SOD1. Here, we show that MIF inhibits mutant SOD1 nuclear clearance when overexpressed in motor neuron-like NSC-34 cells. In addition, MIF alters the typical SOD1 amyloid aggregation pathway in vitro, and, instead, promotes the formation of disordered aggregates, as measured by Thioflavin T (ThT) assay and transmission electron microscopy (TEM) imaging. Moreover, we report that MIF reduces the toxicity of misfolded SOD1 by directly interacting with it, and that the chaperone function and protective effect of MIF in neuronal cultures do not require its intrinsic catalytic activities. Importantly, we report that the locked-trimeric MIFN110C mutant, which exhibits strongly impaired CD74-mediated cytokine functions, has strong chaperone activity, dissociating, for the first time, these two cellular functions. Altogether, our study implicates MIF as a potential therapeutic candidate in the treatment of ALS.

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A47 Determination of the role of CorticoSpinal Motor Neurons in ALS onset and progression

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Joint degeneration of CorticoSpinal Motor Neurons (CSMN) and Bulbar/Spinal Motoneurons (BSMn) leads to ALS diagnostic and gives the disease its particularly lethal character, compared to diseases in which only one of these neuronal populations is affected. Recent genetic, functional, and histopathological studies carried on ALS patients suggests that the disease may originate from the cerebral cortex and spread to its projection targets, including the brainstem and spinal cord, according to the so-called â€œcorticofugal hypothesisâ€Ð. In this context, it appeared necessary to unravel the role of CSMN, as appointed intermediaries between the cerebral cortex and the brainstem and spinal cord. To evaluate the contribution of CSMN to ALS, we generated a mouse model that overexpress a mutant of the Sod1 gene, a condition sufficient to develop ALS, but that totally lacks the CSMN and a direct connection between the cerebral cortex and the spinal cord. This was obtained by crossing Sod1G86R mice [1] to the Fezf2 KO mice [2] that lack the gene encoding a transcription factor necessary for the generation of CSMN during development [3]. We generated 4 groups of animals, Fezf2 KO/Sod1G86R; WT/Sod1G86R, and controls Fezf2 KO/WT; WT/WT, and are currently running a survival and motor behavioural study. We are also generating additional animals for histology and molecular biology at 3 relevant time points of disease progression: pre-symptomatic, early and late symptomatic. Experiments are ongoing to determine how, in rodents, CSMN contribute to ALS onset and progression, and whether they may participate to SMN degeneration. [1] Ripps ME, Huntley GW, Hof PR, et al. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis, PNAS, 92, 689-693 (1995). [2] Hirata, T. et al. Zinc finger gene fez-like functions in the formation of subplate neurons and thalamocortical axons. Dev. Dyn. 230, 546â€“556 (2004). [3] Molyneaux, B. J., Arlotta, P., Hirata, T., Hibi, M. & Macklis, J. D. Fezl is required for the birth and specification of corticospinal motor neurons. Neuron 47, 817–831 (2005).

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A48 UBA1/GARS-dependent pathways drive sensory-motor connectivity defects in spinal muscular atrophy

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Deafferentation of motor neurons as a result of defective sensory-motor connectivity is a critical early event in the pathogenesis of spinal muscular atrophy (SMA), but the underlying molecular pathways remain unknown. We show that restoration of ubiquitin-like modifier- activating enzyme 1 (UBA1) was sufficient to correct sensory-motor connectivity in the spinal cord of SMA mice. Aminoacyl-tRNA synthetases, including GARS, were identified as downstream targets of UBA1. Regulation of GARS by UBA1 occurred via a non-canonical pathway independent of ubiquitylation. Dysregulation of UBA1/GARS pathways in SMA mice disrupted sensory neuron fate, phenocopying GARS-dependent defects associated with Charcot Marie Tooth disease (CMT). Sensory neuron fate was corrected following restoration of UBA1 expression and UBA1/GARS pathways in SMA mice. We conclude that defective sensory-motor connectivity in SMA results from perturbations in a UBA1/GARS pathway that modulates sensory neuron fate, thereby highlighting significant molecular and phenotypic overlap between SMA and CMT.

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A49 Mitochondrial abnormalities & disruption of the neuromuscular junction precede the clinical phenotype & motor neuron loss in hFUSWT transgenic mice

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FUS (fused in sarcoma) mislocalization and cytoplasmic aggregation are hallmark pathologies in FUS-related amyotrophic lateral sclerosis and frontotemporal dementia. Mechanistic hypotheses have largely focused on a loss of nuclear function in transcriptional regulation and splicing. Recent studies describe the presence of FUS in dendritic spines, implying a role in regulating mRNA axonal transport and local translation at the synapse. Here, we report that FUS is highly abundant at the pre-synaptic terminal of the neuromuscular junction (NMJ), suggesting an important function at the peripheral synapse. We have previously reported a progressive and ultimately fatal motor deficit due to motor neuron degeneration in transgenic mice overexpressing human wild-type FUS. Now, we report the earliest ultrastructural changes in the pre-synaptic terminals including mitochondrial abnormalities at postnatal day 6, which are more pronounced at P15 together with dramatic loss of synaptic vesicles and synaptophysin protein. However, these changes occur in the presence of abundant FUS and without significant loss of spinal cord motor neurons, indicating a peripheral toxic gain of function and a 'dying-back' axonopathy. These results implicate a novel and important function of FUS at the NMJ, and challenge the 'loss of nuclear function' hypothesis for disease pathogenesis.

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A50 The Crym-CreERT2 mouse line to study the role of corticospinal motor neurons in ALS

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While evidence of combined degeneration of the spinal and bulbar motoneurons together with the corticospinal motor neurons (CSMN) is required to diagnose Amyotrophic Lateral Sclerosis (ALS), preclinical studies have mostly concentrated on the spinal motoneurons, leaving aside the CSMN and their contribution to ALS onset and progression. This seeming disinterest may arise, at least partly, from a lack of tools to selectively label CSMN within the cerebral cortex and assess their function or dysfunction in animal models. We generated a Crym-CreERT2 mouse line, in which the inducible CreERT2 recombinase gene is knocked-in within the endogenous Crym locus, downstream of the gene coding sequence. Crym gene was selected for its almost exclusive expression within the cortical layer V where CSMN reside, and its absence of expression from the spinal cord or the skeletal muscle. The design of the mouse line enables to maintain the endogenous Crym expression, while driving a spatial and temporal expression CreERT2 similar to that of Crym, and allowing for recombination at any time point of interest upon Tamoxifen injection. To test whether Crym-CreERT2 expression reproduces Crym endogenous expression, we first conducted immunofluorescence to revealed CRE protein expression, and demonstrated its co-localization with typical cortical layer V markers. Next, we crossed the Crym-CreERT2 mice with the Rosa-tdTomato reporter mice and observed 1) that tdTomato-positive cells were detected only in Tamoxifen-(Tam-) injected animals; 2) that tdTomato-positive cells recapitulated Crym expression; 3) that tdTomato-positive cells co-expressed typical corticofugal markers such as CTIP2. Together, the data demonstrate that CRE expression recapitulates Crym expression, with an almost exclusive expression by the subcerebral projection neurons of the cortical layer V. Retrograde labelling of the CSMN is on-going to further verify that Crym-CreERT2 mice allow recombination within the CSMN. Importantly, no tdTomato expression could be detected in the spinal cord of Tam-injected animals. Altogether, the data indicate that recombination can be achieved within layer V excitatory projection neurons that include CSMN and suggest that the Crym-CreERT2 mice represent a potentially useful tool to start investigating the role of CSMN in ALS, along with the cell-autonomous versus non-cell-autonomous effects of mutant transgene expression within this clinically relevant neuronal populations.

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A51 Oxidation resistance 1 (OXR1) is neuroprotective in cellular and animal models of amyotrophic lateral sclerosis

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Oxidative stress is a known pathological feature of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Therefore, it is essential to identify proteins that are protective against oxidative stress and that could be utilized as potential new therapeutic targets for these disorders. We are studying a novel protein, oxidation resistance 1 (OXR1), which we have shown to be neuroprotective against oxidative stress both in vitro and in vivo. In addition, we have demonstrated that OXR1 may be involved in the pathological processes that lead to ALS; OXR1 protein levels are increased in postmortem spinal cords from patients with ALS, and Oxr1 binds to the ALS-associated proteins TAR DNA-binding protein 43 (TDP-43) and RNA-binding protein FUS/TLS (FUS). Significantly, over-expression of Oxr1 in vitro can reduce cellular pathogenic features such as protein aggregation and mitochondrial defects that are associated with ALS mutations in TDP-43 and FUS. In a subsequent study, we also demonstrated that over-expression of Oxr1 in vivo in neurons of hSOD1(G93A) mice extended survival, reduced motor neuron degeneration and improved motor coordination. In the current study, we are using a combination of in vivo and in vitro approaches to investigate whether Oxr1 would be neuroprotective in the context of a new mouse model of TDP-43(M337V)-associated ALS. We have shown that genetically increasing neuronal Oxr1 levels improved motor coordination and muscle strength, and reduced neuromuscular junction degeneration and muscle denervation in the TDP-43(M337V) mouse. Using motor neuron cultures from TDP-43(M337V) mice, we have demonstrated that Oxr1 significantly reduces the aberrant cytoplasmic mislocalisation of TDP-43, a key pathogenic hallmark of ALS. We also confirmed that Oxr1 and TDP-43 interact in vivo and we are currently investigating the functional mechanisms driven by this interaction. Thus, our data suggest that OXR1 is a potent protective factor that could be used against all forms of ALS associated with TDP-43 pathology.

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A52 Genetic and pharmacological effects of mGlu5 receptor blockade in the SOD1G93A mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of motoneurons (MNs). Although the etiology is not completely understood and has been ascribed to numerous causes, glutamate(Glu)-mediated excitotoxicity is still one major factor for MNs death (PMID: 26584004). At present there is no effective cure thus focused drug therapy are definitely needed. In this scenario Group I metabotropic glutamate receptors (mGluR1 and mGluR5) represent potential targets, since they are actively involved in the regulation of cellular processes altered in ALS (PMID:18617894, PMID: 22072391, PMID:22634363). We demonstrated that knocking-down mGluR1 significantly prolongs survival and ameliorates the clinical progression in the SOD1G93A mouse model of ALS (PMID:24361555). Based on our results, we planned to investigate the role of mGluR5 in ALS. To this aim, we exploited two in-vivo experimental approaches testing the effects of: i) the genetic ablation of mGluR5 and ii) the pharmacological treatment with an mGluR5 negative allosteric modulator, in SOD1G93A mice. We first generated double mutant mice carrying the SOD1G93A mutation and a partial or a total (KO) mGluR5 deletion (SOD1G93AmGluR5+/-;SOD1G93AmGluR5-/-). Both SOD1G93AmGluR5+/- and SOD1G93AmGluR5-/- mutant mice showed a shift of the pathology onset and a significant prolonged survival probability, measured by the Kaplan Meier analysis. The results were paralleled by a significant spinal cord MNs preservation, a decreased astrocyte and microglia activation and by a normalization of the excessive Glu release in SOD1G93AmGluR5+/- vs. age matched SOD1G93A mice. In order to translate the mGluR5 genetic ablation into a pharmacological treatment, we administered SOD1G93A mice with the orally bioavailable mGluR5 negative allosteric modulator CTEP (PMID: 25565255). We start the treated at 90 days of life (4 mg/kg every 24h) and we analyzed the clinical progression and the survival probability. The CTEP treatment significantly increased the survival probability and slowdown the clinical progression of the pathology. Of note, the pharmacological treatment turned out to be more effective in SOD1G93A female compared to male mice. Overall, these data suggest that the genetic ablation or the pharmacological blockade of mGluR5 has a significant impact in-vivo on ALS clinical outcome and provide a rationale for pharmacological approaches based on the selective block of Group I mGluRs.

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A53 Characterization of a novel FUS zebrafish model to study the ALS-FTD spectrum

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FUS is an RNA-binding protein involved in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Altered FUS nuclear localization and cytoplasmic FUS aggregates are found in ALS patients carrying FUS mutations, as well as in a subset of FTD patients. It is estimated that mutations in FUS account for 5% of familial ALS and more than 60% of cases with FUS mutations show disease onset before 45 years of age, with many juvenile ALS cases presenting with disease onset in late teens and early 20's. Since the discovery of the gene, a number of animal models have been developed to study its mechanisms. But among all these studies, no mutant line in zebrafish has been clearly described with phenotypic features associated to ALS-FTD. The objective of this work here is to characterize a novel FUS zebrafish model to study the ALS-FTD spectrum. For this, we started to score for possible phenotypes in a new undefined FUS zebrafish mutant line. We show that the FUS homozygous deletion mutants have a survival deficit compared to the wild types and the FUS heterozygous mutants. We also notice that the FUS mutation leads to a severe motor phenotype with the homozygous mutants being unable to swim efficiently, either in stimulus-induced locomotion or in spontaneous locomotion tests. To further characterize this phenotype, we looked at the motoneuron morphology in this model and found that the FUS mutation causes an excessive elongation of the axons. It was also observed that the mutation affects the muscle structure of the embryos and has an effect on the formation of the neuromuscular junction. Following the description of the phenotype, we are also working on a possible rescue through injection of FUS RNA into one-cell stage eggs. To extend our study, we plan to strengthen the link between FUS and other proteins implicated in ALS-FTD, TDP-43 and TAU. To define the relationship between these proteins, we are currently developing and characterizing deletion mutant lines of FUS and TDP-43 using CRISPR/Cas9 technology to determine phenotypic features associated with loss of function of these factors. We will then cross these animals with mutant TAU transgenic zebrafish lines. Finally, bioactive compounds will be tested to determine the neuroprotective properties of these drugs and to define novel therapeutic strategies for ALS and FTD. Thus, these novel zebrafish models will lead to a better understanding of the pathogenic mechanisms that occur in ALS-FTD.

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A54 Translating ribosome affinity purification from C9orf72- ALS/FTD patient-derived iPS motor neurons

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Background: The intron 1 C9orf72 hexanucleotide repeat expansion is the most common cause of amyotrophic lateral sclerosis (ALS) in both sporadic and familial patients. Transcriptomic analysis of patient-derived iPS motor neurons is challenging due to multiple sources of experimental variance, such as the presence of up to 30% of cells in culture being of unknown identity. Aims: The aim of this project is to use translating ribosome affinity purification (TRAP) to isolate RNA from translating polysomes in a pure population of motor neurons to achieve more accurate analysis of the translatome profile of this disease model. Comparing the translatome profiles of patient-derived iPS motor neurons to isogenic CRIPSR/Cas9-corrected lines will allow a more accurate estimation of the effect the C9-repeat expansion has on has molecular pathology. Methods: We cloned a TRAP construct with a large ribosomal subunit protein, RPL22, fused with a c-myc-FLAG affinity tag, and bicistronic enhanced green fluorescent protein gene, under a choline acetyl transferase (ChAT) promoter. Magnetic beads coated with anti-tag antibodies were used to capture tagged RPL22 subunit-containing polysomes from iPS motor neuron lysates and RNA extraction was used to purify motor neuron-specific mRNA from C9/ALS patient lines and CRISPR/Cas9-edited patient control lines. After quality control steps, RNA will be sent for sequencing to identify key pathways associated with early changes of neurodegeneration in C9orf72 motor neurons. Results: Immunofluorescence of fixed control and patient iPS motor neurons, transduced with high concentrations of ChAT:TRAP lentiviral particles, has shown up to 100% co-expression of the eGFP reporter and ChAT. TRAP purification has revealed these RNA populations to be enriched with ChAT and RPL22-c-myc-FLAG transcripts compared with motor neurons transduced with mammalian TRAP constructs and corresponding input fractions, respectively. Immunoblotting showed the expression of both endogenous RPL22 and exogenous tagged RPL22 in iPS motor neurons. Optimisation of the TRAP protocol at several steps has been crucial to reliably obtain this enriched RNA. Conclusions: We are currently carrying out differentiations to obtain RNA samples for sequencing. Analysis of the C9/ALS patient translatome, in conjunction with proteomic analysis is anticipated to overcome some of the problems currently associated with the variation in transcriptomic outputs from iPSC models in ALS.

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A55 Glutamate receptor properties and intracellular calcium dynamics of ALS iPSC derived motor neurons

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The neurodegenerative disease amyotrophic lateral sclerosis (ALS) is characterized by profound loss of spinal motor neurons (MNs) in the ventral horn. Until now only one drug, Riluzole is approved in the EU that minimally extends life expectation, presumably by blocking glutamatergic transmission. However, the excitotoxicity hypothesis that holds excessive glutamatergic transmission and consequently toxic intracellular calcium levels responsible for the observed neurodegeneration has been challenged recently by independent observations. Still, there is sufficient evidence that the glutamatergic transmission system as well as imbalanced calcium homeostasis play a crucial role in ALS related MN death. In the present study we therefore aimed to unravel multiple aspects of the glutamate receptor system in induced pluripotent stem cell (iPSC) derived MNs and also to investigate key aspects of intracellular calcium dynamics. IPSC based disease models of ALS provide an optimal platform to study molecular and cellular disease mechanisms. Following MN differentiation of iPSC from healthy controls and patients with mutations in the TARDBP, FUS, SOD1 or C9Orf72 genes, we performed calcium imaging experiments and real time quantitative PCR. Additionally we used isogenic controls of one iPSC cell line with a mutation in the SOD1 gene and one with a mutation in the FUS gene. Upon application of compounds stimulating either AMPA, NMDA, kainate or metabotropic glutamate receptors we observed only slight differences in the overall number of responding MNs and in the corresponding amplitudes of the responses. QPCR data revealed mutation specific changes in the expression levels of the different glutamate receptors. Furthermore, basal intracellular calcium levels were found to be decreased in iPSC derived MNs with SOD1 mutations. Spontaneously occurring calcium transients were observed with higher frequencies in mutant C9Orf72 MNs. Increase in intracellular calcium levels via addition of thapsigargin, a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) resulted in small differences in the number of responding MNs and in the corresponding amplitudes. Ongoing experiments include immunocytochemical staining of the cell lines with MN markers and qPCR analysis of the expression of MN markers. Our data indicate that different mutations have a different effect on glutamtate receptor properties

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A56 Modulation of the adult SOD1G93A astrocyte phenotype by treatment with exosome-shuttled miRNAs derived from mesenchymal stem cells

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by muscle atrophy and paralysis. ALS is a non-cell autonomous disease that involves also microglia and astrocytes, which promote an inflammatory environment that contributes to motor neuron death. In particular, astrocytes acquire a reactive phenotype able to secrete neurotoxic cytokines. In a previous study we observed that intravenous administration of MSCs in SOD1G93A mice, an animal model of human ALS, prolongs survival probability, ameliorates motor skills and reduces gliosis and inflammation in spinal cord. These beneficial effects were determined by MSC-produced paracrine factors. To understand the mechanisms underlying the effects of MSCs, we studied here the activity of MSC-derived exosome and exosome-shuttled miRNAs on cultured astrocytes prepared from spinal cord of adult SOD1G93A mice. We hypothesized a modulation of astrocyte activation and a reduction of the inflammatory environment surrounding MNs. We observed a significant increase of GFAP and vimentin, markers of astrocytic reactive phenotype, in SOD1G93A compared to WT astrocytes. After treatment with exosomes, the expression of GFAP and vimentin was reduced by 40% and 80%, respectively. We also analyzed the expression of a number of pro- and anti-inflammatory cytokines. The level of IL1Ð, TNFÐ, IL6 was significantly higher in SOD1G93Athan in WT astrocytes. The same as above, exposure of SOD1G93A astrocytes to exosomes resulted in a significant decrease of these cytokines expression, by 65%, 80% and 60%, respectively. We also detected a higher expression of IL10, an anti-inflammatory cytokine in SOD1G93A astrocytes treated with exosomes compared to non-treated SOD1G93A astrocytes. The expression of NLRP3 was increased in SOD1G93A astrocytes compared to WT astrocytes and this increase was reversed (70%) by treatment with exosomes. We tested also 466q and 467f miRNAs, which have been found to be up-regulated un MSCs and present in exosomes. Both miRNAs decreased the expression of IL1Ð and TNFÐ in SOD1G93A astrocytes. These results suggest that exosome-shuttled miRNAs ameliorate the inflammatory state and promote a shift of astrocytes to a neuroprotective phenotype. These data are promising to translate this therapeutic approach in a pre-clinical trial.

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A57 Human induced pluripotent stem cells-derived motor neurons for modelling age-related pathophysiological mechanisms of ALS

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Pathologic phenotype of motor neurons in ALS partially resembles features found during ageing, such as mitochondrial failure, oxidative stress and proteotoxicity. Understanding ALS pathogenesis is hindered by the lack of models that recapitulate the disease accurately; human samples for systematic studies are hardly available and most of the transgenic mice do not completely resemble the human phenotype. In this context Human induced pluripotent stem cells (hiPSCs) represent a real breakthrough for disease modelling. Based in the recent work of several groups, generating bona fide MNs from hiPSCs, we hypothesize that the pathophysiological mechanisms of the disease could be modelled in vitro, including ageing-accelerated pathological patterns in MNs. As a proof of concept, we present changes in nuclear envelope and nuclear structure proteins (e.g nucleoporins and lamins), in the respiration capacity of MNs and their progenitors, its superoxide production and changes in TDP-43 subcellular locations in response to different, age-related, cellular stress stimuli. We hypothesize that the biological effects of these stressors (and host genotypes) can be associated with changes in lipid composition, resembling changes present in ALS nervous system samples. We will present an optimized culture conditions to obtain MNs expressing SMI32, HB9, MAP2 and Tau. The resulting MNs can express ChAT and motor plaque epitopes in cocultures with C2C12 myotubes, overall leading to contraction. Globally, we present a method for generating human MNs in a dish with molecular and functional features of their in vivo counterparts, highlighting the value of this model in disease modelling. We hope that our results will help to understand the pathogenesis of the disease, clarifying the potential role of MNs accelerated ageing.

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A58 Cellular pathways dysregulated by mutant FUS in CRISPR/Cas9 cell models

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Background. A subset of familial ALS cases is caused by mutations in the FUS gene which render the protein prone to partially mislocalise to the cytoplasm. It is believed that both loss and gain of FUS function drive pathology in ALS-FUS cases. Recently, we generated a panel of neuroblastoma cell lines using CRISPR/Cas9 genomic editing. These lines express physiological levels of mutant FUS (deltaNLS) with either one of both copies of the FUS gene edited. Homozygous FUS deltaNLS lines are characterised by prominent cytoplasmic mislocalisation of FUS whereas heterozygous lines have low levels of cytoplasmic FUS. In addition, FUS knockout (KO) cells were produced. Aim. To understand how the extent of FUS mislocalisation to the cytoplasm affects gene expression profiles. Methods. Transcriptomic analysis of three heterozygous and three homozygous FUS deltaNLS cell lines alongside with WT and FUS KO cell lines was performed. Results. Only a minor subset of differentially expressed genes (DEGs) in FUS dNLS cells was consistent with FUS loss of function. GO term enrichment analysis of DEGs revealed that genes decreased both in heterozygous and homozygous FUS deltaNLS cell lines show the enrichment for those involved in synaptic transmission, nervous system development, neuronal differentiation and ion transport. Strikingly, more genes were found to be dysregulated in heterozygous FUS deltaNLS cells compared to homozygous cells, with most of DEGs being upregulated. Functional analysis of DEGs upregulated at least 2-fold in heterozygous and homozygous FUS deltaNLS cells showed a significant enrichment for 'regulation of apoptotic process' and related categories uniquely in heterozygous FUS deltaNLS cells. Discussion and conclusions. Our data indicate that the presence of one copy of the mutant FUS, which is not associated with dramatic cytoplasmic mislocalisation of the protein, is sufficient to drive significant transcriptomic changes and perturb pathways related to cellular death. This suggests that mutant FUS may exert negative effects in the nucleus independent of its gain of function in the cytoplasm. Acknowledgments. The work was supported by Motor Neuron Disease Association (Buchman/Apr13/6096). TS is a Medical Research Foundation fellow. HA is supported by China Scholarship Council/Cardiff University PhD studentship.

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A59 Impaired DNA damage response signaling by FUS- NLS mutations leads to neurodegeneration and FUS aggregate formation

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Amyotrophic lateral sclerosis (ALS) is the most frequent motor neuron disease. Cytoplasmic fused in sarcoma (FUS) aggregates are pathological hallmarks of FUS-ALS. Proper shuttling between the nucleus and cytoplasm is essential for physiological cell function. However, the initial event in the pathophysiology of FUS-ALS remains enigmatic. Using human induced pluripotent stem cell (hiPSCs)-derived motor neurons (MNs), we show that impairment of poly(ADP-ribose) polymerase (PARP)-dependent DNA damage response (DDR) signaling due to mutations in the FUS nuclear localization sequence (NLS) induces additional cytoplasmic FUS mislocalization which in turn results in neurodegeneration and FUS aggregate formation. Our work suggests that a key pathophysiologic event in ALS is upstream of aggregate formation. Targeting DDR signaling could lead to novel therapeutic routes for ameliorating ALS.

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A60 The anterior cingulate cortex in the ALS-FTD spectrum: post mortem MRI-histology correlation

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Introduction The histopathological staging systems based on phosphorylated 43 kDa transactive response DNA binding protein (pTDP-43) proposed in ALS and frontotemporal dementia (FTD) [1] have specifically highlighted involvement of the anterior cingulate cortex (ACC). Atrophy of the ACC has been associated with behavioural variant frontotemporal dementia (bvFTD) [2]. Diffusion tensor imaging (DTI) has the potential to non-invasively detect white matter and grey matter abnormalities associated with disease progression. Objectives To study post mortem histopathological changes in the ACC of ALS-FTD spectrum patient brains in relation to ultra-high field DTI findings. Methods Donated brains from ALS-FTD disease spectrum (n =13) and control (n=3) individuals underwent whole brain 7T MRI and systematic histopathological sampling. Serial sections of the ACC were immunostained and quantitatively analysed with digital microscopy to determine the pTDP-43 load, microglia activation and axonal degeneration and compared to DTI parameters (e.g., fractional anisotropy, mean diffusivity). Results A variable degree of pTDP-43 pathology in ALS and ALS-FTD cases was demonstrated, with the burden of ACC pTDP-43 significantly greater in ALS-FTD compared to ALS cases. Pearson correlation showed no association between the pTDP-43 load and inflammatory changes. Ongoing analysis of other microstructural changes of the ACC in relation to DTI parameters will be presented. Conclusion The degree of pTDP-43 pathology burden in the ACC is strongly linked to the presence of FTD in the ALS spectrum. Correlation with DTI changes in the AAC might then have predictive and prognostic value in vivo. References 1.Brettschneider J, et al Ann Neurol 2013;74:20-38 2.Tan RH, et al Acta Neuropath Communications 2013;1:30 Acknowledgment This project is funded by the Medical Research Council. We acknowledge the Oxford Brain Bank, supported by the Medical Research Council (MRC), Brains for Dementia Research (BDR) (Alzheimer Society and Alzheimer Research UK), Autistica UK and the NIHR Oxford Biomedical Research Centre.

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A61 Safety and efficacy of human embryonic stem cells derived astrocytes following intrathecal transplantation in SOD1G93A and NSG animal models

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ALS is a Motor Neuron (MN) disease characterized by the loss of MNs in the central nervous systems. As MNs die, patients progressively lose their ability to control voluntary movements, become paralyzed and eventually die from respiratory/deglutition failure. Despite the selective MN death in ALS, there is growing evidence that malfunctional astrocyte play a crucial role in disease progression. Thus, transplantation of healthy astrocytes may compensate for the diseased astrocytes. We developed a GMP-grade protocol for generation of astrocytes from human embryonic stem cells (hESC). The first stage of our protocol is derivation of astrocyte progenitor cells (APC) from hESCs. These APC can be expanded in large quantities and stored frozen as cell banks. Further differentiation of the APC yields an enriched population of astrocytes (>90% GFAP+). In vitro, these cells possess the activities of functional healthy astrocytes, including uptake of glutamate, promotion of axon outgrowth and protection of MNs from oxidative stress. A secretome analysis shows that these hESC-derived astrocytes (hES-AS) also abundantly secrete several inhibitors of metalloproteases as well as variety of neuroprotective factors (e.g. TIMP-1&2, OPN, MIF and Midkine). In animal models of ALS, hSOD1G93A high-copy number transgenic mice and rats, intrathecal injections of the hES-AS significantly delayed disease onset and improved motor performance compared to sham-injected animals. A nine-month safety study conducted in NSG animal model under GLP conditions showed that intrathecal transplantation of hES-AS to the cerebrospinal fluid (CSF) is safe. Transplanted hES-AS attached to the meninges along the neuroaxis and survived for the entire duration of the study without expansion or formation of tumors or teratomas. Cell-injected injected mice gained similar body weight as the sham injected group and did not exhibit clinical signs that could be related to the treatment. No differences from the vehicle control was observed in hematological parameters or blood chemistry. These findings demonstrate the feasibility, safety and potential efficacy of intrathecal injections of hES-AS for the treatment of ALS.

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Seminar Room A:

C01 Cognitive impairment in facial onset sensory and motor neuronopathy (FOSMN)

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Facial Onset Sensory and Motor Neuronopathy (FOSMN) is a rare neurodegenerative condition of uncertain pathogenesis which typically presents with a trigeminal distribution sensory disturbance progressively engulfing the head, neck and upper trunk bilaterally, and associated with facial, bulbar and respiratory motor impairment. The handful of reported neuropathological post-mortem analyses have demonstrated neuronal loss within the brainstem nuclei in addition to the cervical dorsal root ganglia and anterior horns. However, TDP-43 inclusion bodies more typical of the amyotrophic lateral sclerosis (ALS) and frontotemporal dementia disease spectrum have been demonstrated in several of these cases, sparking debate as to whether FOSMN may represent an unusual manifestation of ALS. Although not apparently a principal facet of disease presentation, little attention has been paid to the presence of cognitive impairment in FOSMN and, indeed, has even been considered as an exclusion criterion for the condition.
Here we report characteristics of cognitive difficulties in 3 patients with FOSMN which were manifest 12-24 years into disease duration. All complained of poor anterograde memory for recent events or conversations. Two patients developed mild frontal dysexecutive features including apathy, reduced empathy, poor organisational skills, impaired task switching and reduced verbal fluency. The remaining patient experienced frequent visual hallucinations and confused nocturnal wandering. Interestingly, two subjects were found to have REM sleep behaviour disorder on overnight sleep studies which would perhaps broadly correspond to the ponto-medullary region of degeneration in FOSMN but would instead classically herald one of the synucleinopathies. Furthermore, two patients additionally developed mild-moderate high frequency sensorineural hearing loss documented from their mid-50s, which could imply involvement of central auditory circuitry or plausibly be incidental presbyacusis. These cases expand the phenotype of FOSMN and strongly suggest that cognitive deficits are encountered, particularly in well-established cases.

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C02 Amyotrophic lateral sclerosis related cognitive deficits are a marker of localized TDP-43 cerebral pathology

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease characterized by degeneration of both upper and lower motor neurons. In addition to motor symptoms, many patients also exhibit cognitive deficits particularly in fluency, executive function and language. We performed an in-depth neuropathological analysis of 27 ALS patients who had undergone cognitive testing using the Edinburgh Cognitive ALS Screen (ECAS) during life with the aim of determining the pathological correlate of these ALS related cognitive impairments. More specifically this study aimed to (i) determine whether cognitive impairment as detected by the ECAS was a good in vivo predictor of extra-motor pathology in ALS and (ii) determine whether the profile of cognitive impairment would relate to a specific regional distribution of pathology in the frontal and temporal lobes. 11 individuals were identified as having cognitive impairment (1 executive dysfunction, 6 language dysfunction, 2 fluency dysfunction and 2 exhibiting deficits in all 3 domains). In our analysis, all patients demonstrating cognitive impairment with the ECAS had TDP-43 pathology at post-mortem in extra-motor brain regions (positive predictive value of 100%). The ECAS also predicted TDP-43 pathology with 100% specificity in brain regions associated with executive function, language and fluency domains. We also detected a subgroup of individuals with no clinical manifestations of cognitive dysfunction (normal ECAS), despite having substantial TDP-43 pathology at post-mortem. This group displayed differential spatial expression of a chaperone protein, called clusterin, known to protect against the intracellular proteotoxicity associated with TDP-43 accumulation. Our analysis therefore indicates that the ECAS is a valid predictor of regional TDP-43 pathology at post-mortem and highlights the utility of ECAS in accurately assessing the pathological burden of disease in patients with ALS.

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C03 Neuropsychiatric symptoms in MND patients and their family members

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Introduction: Cognitive and behavioural changes occur in approximately 50% of people living with motor neurone disease (MND) and include increased apathy, stereotyped behaviours, executive dysfunction, disinhibition and impaired social cognition (1). Evidence also suggests there is an increased risk of depression, neurotic disorders and addiction both prior-to and in the year following a diagnosis of MND (2) and that patient's family members (FM) experience more neuropsychiatric disorders, particularly psychosis, suicidal behaviour (3) and autism (4). Objectives: We examined the frequencies of neuropsychiatric symptoms, cognitive impairment and behaviour change in MND patients and their FM compared to the general population and how these symptoms related to cognitive and behavioural impairment. Methods: In Scotland, MND patients and their first and second-degree FM were recruited through the Scottish National MND Register. Healthy controls (HC) and their FM were recruited through the University of Edinburgh Volunteer Panel. Questionnaires measuring symptoms of neuropsychiatric disorders were administered. Probands completed a brief cognitive screen and where possible, an informant provided behavioural information. Results: Sixty MND patients and 36 MND-FM along with 60 HC and 17 HC-FM participated. At assessment, MND-FM reported more symptoms of anxiety (3.36 4.29 vs .06 1.98, t(51)=2.67, p<0.05) and OCD (10.78 9.30 vs 5.65 4.00, t(51)=2.17, p<0.05) compared to HC-FM. MND patients and MND-FM were at increased risk of mania (OR=0.33, 95%CI -2.12 to -0.16 and OR=14.32, 95%CI 0.92 to 5.61). Cognitive and/or behaviour impairment was evident in 30 (50%) MND patients. Symptoms of anxiety were higher in those with impairment (3.14 3.79) than without (1.04 1.75, t(40)=2.68,p<0.05). Symptoms of autism were higher in FM of impaired MND patients than FM of unimpaired MND patients (19.79 4.39 vs 16.31 4.91, t(24)=2.06,p<0.05) Conclusion: Symptoms of neuropsychiatric disorders are uncommon in MND patients however MND-FM experience higher rates of anxiety, OCD and mania. References: 1. Goldstein LH, & Abrahams S Lancet Neurol 2013; 12:368-380 2. Longinetti E, Mariosa D, Larsson H et al Neurol 2017; 578-585 3. Bryne S, Heverin M, Elamin M et al Ann Neurol 2013; 74:699-708 4. OBrien M, Burke T, Heverin M et al JAMA Neurol 2017; 1425-1430

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C04 Emotional apathy and awareness in frontotemporal dementia

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Background: As a prevalent symptom in neurodegenerative disease, apathy is composed of different subtypes of demotivation. ALS is characterised by Initiation apathy (demotivation towards self-generated thoughts/actions), whereas Alzheimerâ€™s disease (AD) shows global apathy over all subtypes with a specific lack of awareness. As ALS and frontotemporal dementia (FTD) exist on a spectrum, it is important to determine the apathy profile in FTD and its association with levels of awareness. Objectives: The aim was to determine the profile and awareness of apathy subtypes in FTD compared to other dementias, such as AD, and an ALS patient group. Methods: 17 FTD patients, 28 AD patients and 20 healthy controls (and their informants/carers) were recruited. A supplementary 30 ALS patients (and their carers) were included from previous research. Groups were matched for age, education and gender. Participants and informant/carers completed- Dimensional Apathy Scale (DAS), assessing Executive, Emotional and Initiation Apathy, Apathy Evaluation Scale and Geriatric Depression Scale. Group comparisons were performed using Bonferroni corrected Kruskal-Wallis tests with Mann-Whitney U post hoc tests. Results: Both FTD and AD patients scored significantly higher than controls on all apathy subtypes (p's<0.01), showing global apathy. FTD patients scored significantly higher only on Emotional apathy compared to AD patients ($p<.05$). When examining awareness (self-rated and informant/carer-rated score discrepancy), FTD patients showed a significantly higher awareness deficit on only Emotional apathy than controls ($p<.01$) and AD patients ($p<.05$). Supplemental comparison showed significantly higher Initiation apathy for both FTD ($p<.001$) and ALS ($p<.01$) patients compared to controls, with no significant difference between FTD and ALS patients. Executive and Emotional apathy were significantly higher in FTD patients compared to ALS patients (p's<.001), with subtype-specific awareness deficit in FTD patients. Conclusions: A higher indifference and emotional neutrality (Emotional apathy), with an awareness deficit, seem to typify the apathy profile in FTD. Increased Emotional and Executive (demotivation to plan, organise and attend) apathy further distinguishes FTD from ALS, while both diseases showed Initiation apathy. This has implications for development of interventions specific to apathy profiles and further research should explore these on the ALS-FTD spectrum.

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C05 The profile of language changes in Amyotrophic Lateral Sclerosis: results from a population-based study of incident cases

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Background: The neuropsychological profile in ALS is now known to be highly heterogeneous, extending beyond executive dysfunction and behavioural change. Reports of language decline in ALS exist, and the revised Strong Criteria for the diagnosis of frontotemporal cognitive and behavioural syndromes now includes language as one of the two core domains to diagnose ALS with cognitive impairment (ALSci). Nevertheless, most studies assessing language in ALS are characterized by small prevalent samples, and the profile of language changes in incident cases is currently unknown. Objective: This study explored the profile of language changes in an incident population-based sample of ALS patients. Methods: 138 patients diagnosed from December 2014 to August 2017 in the Irish National ALS Clinic were recruited, and assessed within the first year of diagnosis on a comprehensive battery of language. Performance was compared to a healthy control sample (n=100), matched by age (p=.534), gender (p=.483), education (p=.098) and premorbid IQ (p=.300). Results: 19 out of the 138 patients (14%) met criteria for ALS-FTD. Language performance was compared between non-demented ALS patients (n=119) and healthy controls. These two samples were also matched by age (p=.300), gender (p=.502), education (p=.094) and premorbid IQ (p=.621), as well as current intellectual ability (p=.108). Non-demented ALS patients performed significantly lower on measures of Visual Lexical Decision (p=.008), Semantic Verbal Fluency (p=.020), Word Spelling (p<.0001), Word Reading (p=.027), Word Naming (p<.0001) and Sentence-Picture Matching for both auditory (p<.0001) and written protocols (p<.0001). No significant differences were observed on measures of Auditory Lexical Decision (p=.185) and Word-Picture Matching (p=.066). When mean performance was analysed for patients meeting criteria for ALSci (30%), only measures of Word Spelling, Word Naming and Sentence-Word Picture Matching were impaired. 9% met criteria for 'ALSci language impairment' with no evidence of executive dysfunction. Conclusions: Language changes are already present during the first year of diagnosis in ALS. These changes occur as a result of frontotemporal network disruption, and are independent of executive dysfunction to a degree. Longitudinal follow-up of this cohort will indicate how these changes advance with disease progression. Acknowledgements: This work is funded by the Motor Neuron Disease Association (MNDA).

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C06 The relationship between apathy subtypes, quality of life and caregiver burden in amyotrophic lateral sclerosis – work in progress

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Background: Apathy is the most prevalent and debilitating behavioural symptom for people living with amyotrophic lateral sclerosis (ALS) and has been found to have a negative impact on quality of life and caregiver burden. Apathy is characterised by a lack of motivation in three areas: a) being unmotivated to plan/organise/attend to finishing tasks (executive apathy), b) not thinking of new ideas (initiation apathy) and c) being indifferent to what is going on around them (emotional apathy). The Dimensional Apathy Scale (DAS) is used to assess these different apathy subtypes, independent of physical disability, and has shown a characteristic apathy profile in ALS. However, the impact of these apathy subtypes on patients' everyday lives and that of their carers has not been explored. Objective: To determine the extent to which different apathy subtypes are associated with quality of life and caregiver burden. Methods: 60 people with ALS and 60 of their carers will be recruited from Scotland and England and will take part in a 1-hour interview. This interview will aim to assess patients' apathy profile (DAS), cognitive functioning and behaviour change (Edinburgh Cognitive and Behavioural ALS Screen), mood (Patient Health Questionnaire-9, Generalised Anxiety Disorder Questionnaire-7), emotional lability (Emotional Lability Questionnaire), quality of life (ALS Specific Quality of Life Instrument-20) and functional disability (ALS Functional Rating Scale-Revised). The caregiver assessment will also look at burden and wellbeing (Carer Experience Scale, Zarit Burden Interview-Short Form, ICEpop CAPability Measure for Adults). Results: Correlational analyses will be used to explore the relationship between the different apathy subtypes, quality of life and caregiver burden. These will form the preliminary results from the baseline visit of the larger longitudinal study. Comparative analyses will also be performed between patients with different apathy profiles, to further elucidate the differences in wellbeing.
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C07 Stage of prolonged survival with riluzole treatment in patients with amyotrophic lateral sclerosis: A retrospective analysis

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Background: Riluzole is the only drug to prolong survival for amyotrophic lateral sclerosis (ALS), providing a 25% reduction in mortality at 12 months. A key question is whether the survival benefit occurs at an early stage of disease, late stage, or is spread throughout the disease course. To address this question, we used clinical staging to re-analyse the original dose-ranging clinical trial of Riluzole. Methods: Patients with probable or definite ALS as defined by the El Escorial criteria were eligible for the original trial. For this analysis, King's clinical ALS stage was estimated from the electronic case record data of the Modified Norris Scale, Medical Research Council scale for muscle strength, vital capacity, and gastrostomy insertion. A chi-squared test was used to test the independence of stage at trial enrolment and treatment group. Kaplan-Meier product limit distribution was used to test the transition from each stage to subsequent stages. Sensitivity analyses were carried out by combining treatment groups, using alternative strategies to stage, stratifying by stage at trial enrolment, and using multi-state outcome analysis of treatments (MOAT). Findings: All 959 participants from the original trial were analysed, with four Riluzole treatment groups, 50mg (n=242), 100mg (n=237), 200mg (n=236) and placebo (n=244) daily. Clinical stage at enrolment was not significantly different between treatment groups (P = 0.22) . Time in Stage 4 was prolonged for those on Riluzole compared with placebo, confirmed on Kaplan-Meier analysis (P = 0.037). Combining treatment groups and stratifying by stage at enrolment showed a similar result (P = 0.006), as did analysis with MOAT. Time in Stages 2 (P = 0.83) or Stage 3 (P = 0.88) was not significantly altered by treatment with Riluzole. Interpretation: In this post-hoc analysis, we have shown that Riluzole prolongs survival in the last clinical stage of ALS; this finding needs to be confirmed in a prospective study, and treatment effects at Stage 1 still need to be analysed. The stage at which benefit occurs is important for counselling of patients before starting treatment. Staging should be used in future ALS clinical trials to assess the stage at which survival benefit occurs, and a similar approach could be used in other neurodegenerative diseases. Funding: Funding was provided by NIHR Maudsley Biomedical Research Centre, The EU Joint Programme on Neurodegeneration (JPND), and the K

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C08 What do people living with ALS in Ireland think about dysphagia and what do they want from dysphagia-related health services? A qualitative study

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Introduction: There are around 350 people living with ALS in Ireland at any one time. Dysphagia is generally reported to interfere with the quality of life of people with ALS, however, little is known about how people living with ALS (both people diagnosed with ALS and their caregivers) understand and experience dysphagia. Research aims: To explore the experiences of dysphagia in ALS from the perspectives of people with ALS (PwALS) and their caregivers in order to investigate: how they understand dysphagia, how dysphagia impacts their lives, their coping strategies in relation to dysphagia, and their experiences of health-services received to manage dysphagia. Methods: 10 PwALS and 10 caregivers from South-West of Ireland participated in multiple interviews (n=58) supported by observations. All PwALS had a confirmed diagnosis of dysphagia and had received professional recommendations to manage dysphagia, had no cognitive impairment, were able to communicate at a sentence level (verbally or non-verbally) and were at least 2 months post ALS diagnosis. The caregivers were regularly involved in diet preparation and/or feeding. A pilot study was completed. Data analysis: Interpretative Phenomenological Analysis (Smith et al. 2009) where and ideographic approach was followed by a cross-case analysis of the two groups. The findings were interpreted from a Speech and Language Therapy perspective. Results: A difference was noted between participants' own perception of dysphagia and their clinical presentation. The decision-making process with regard to non-oral feeding was reported to be traumatic for the majority of participants, particularly, if they felt they were receiving contradictory advice from professionals. Many factors were identified which could potentially influence a decision to consent to a gastrostomy. A changed perception of food was observed in both groups. PwALS aimed to manage dysphagia alone, however, the caregivers wished for increased professional support. Discussion: PwALS and their caregivers understand dysphagia differently and may have different expectations regarding dysphagia management. The recognition of swallowing difficulties in oneself seems to be a lengthy process which does not always mirror the process of professional diagnosis. This may influence individualâ€™s attitudes towards professional recommendations and their engagement with health services. Professionals should look beyond the swallowing impairment alone.

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C09 Causes of death in amyotrophic lateral sclerosis. Results from the Rhineland-Palatinate ALS registry

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Background: Amyotrophic lateral sclerosis (ALS) is associated with an increased mortality. Knowledge of possible causes of death could lead to an individualization of the palliative treatment concept and result in a differentiated palliative treatment pathway. Objective: Analysis of the various causes of death in a prospective population-based German cohort of ALS patients. Methods: Analysis of data of the Rhineland-Palatinate ALS registry in which newly diagnosed patients identified between October 2009 and September 2012 were prospectively enrolled and followed up at regular intervals. The causes of death were elicited based on information provided by the attending physicians, family members and by means of death certificates registered by the regional health authorities in Rhineland-Palatinate. Results: Out of 200 ALS patients registered 148 died between register initiation and the end of follow-up on 30 September 2015 (78 males and 70 females). The most frequent cause of death was respiratory failure as a consequence of weakness of respiratory muscles (n = 91, 61%). Less frequent causes of death were pneumonia (n = 13, 9%), terminal cachexia (n = 9, 6%) and cardiovascular disorders including sudden death (n = 9, 6%). Cases of suicide were rare (n = 3, 2%) as were deaths due to concurrent diseases (n = 2). In 21 cases (14%) the exact cause of death could not be clarified. Differences in the causes of death showed a weak association with the ALS phenotype. Respiratory failure was the cause of death in all patients with a respiratory phenotype and in 78% of patients with flail arm syndrome. In ALS patients with additional frontotemporal dementia (n=16) death due to respiratory failure was less frequent (33% vs. 65%) while pneumonia was more frequent (27% vs. 7%). Conclusion: Respiratory failure was the most frequent cause of death in our cohort of ALS patients. In contrast, pneumonia and nutritional disorders were minor important causes of death. The ALS phenotype expression of ALS might in part be supportive to predict the cause of the prospective death. Differentiation of ALS phenotypes is an important basis for patient counseling on the prospective process of dying and for the determination of an individual palliative concept.

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C10 Flail arm syndrome – diagnostic challenge: A case report

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Introduction: Flail arm syndrome (FAS) is an atipical presentation of amyotrophic lateral sclerosis (ALS) which is characterized by progressive, predominantly proximal, weakness and atrophy of the upper limbs, without involvment of the lower limb, bulbar or respiratory muscles. Diagnosis may be difficult, particularly at the beginning of the disease. Prognosis is significantly better than typical ALS. Case report: A 64-year old female patient gradually developed weakness and atrophy of right hand which spread to proximal, and after some months at the left side also. She experienced fasciculation very rarely. After six months from the beginning of symptoms she was admitted to hospital. The neurological examination: moderate atrophy and muscle weakness of upper limbs, slightly more distal then proximal and on the right side. EMNG: moderate chronic neural lesion with fasciculation at both arms. Changes are slightly more distal and on right side. Examination in other areas (bulbar, thoracic, lumbosacral) was normal. Laboratory results including tumorous markers and anti-GM1 antibodies were normal. Additional tests were used to exclude possible mimics such as cervical myelopathy, syringomyelia, cervical root lesions and motor neuropathy. We suspected at FAS, but considered UL-ALS, PMA and motor neuropathy. She was treated with intravenous immunoglobulin (IVIg) for five consecutive days (0.4 g/kg/day) and after that with two single dosis every 6 weeks. After 6 and 12 months, her clinical and EMG examination showed slightly worsening. She was diagnosed with FAS. Simptoms and EMG signs were confined at upper limbs. She did not have difficulties with lower limbs, no upper motor neuron signs, and no signs of motor neuropathy. Negative response to the application of IVIg does not support diagnosis of motor neuropathy. Conclusion: Diagnosis of FAS towards UL-ALS, PMA and motor neuropathy may be difficult, particularly at the beginning of the disease. It is important to recognise these diseases because we can threat patients with motor neuropathy, and prognosis patients with FAS is more favorable than prognosis of UL-ALS and PMA. Key words: flail arm syndrome, amyotrophic lateral sclerosis, motor neuropathy

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C11 A first year in life of Zagreb - ENCALS centre

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Department of Neurology, University Hospital Centre Zagreb was constituted in 1921. and has a long-standing tradition of excellent medical care. Division for neuromuscular diseases is the Referral Centre for Neuromuscular diseases and Clinical Electromyoneurography of Ministry of Health, Republic of Croatia and has been providing multidisciplinary services to patients for years (dysphagia assessment and management including PEG when required, speech and cognitive assessments and support, pulmonary function assessments and initiation of non-invasive ventilation when required) but it is only last year our ALS centre was officially formed and joined ENCALS. Although one could look at this as a minor event in a large hospital centre it has turned out to be a very inspiring one. In just a year our centre has started to think not just about continuing to provide up-to-date medical care, treatment and support for our patients but about actively seeking ways to contribute to the pool of knowledge about motor neuron disease. To list some of the activities we have undertaken in last year: - we have started a clinic based registry of ALS patients - we have created a blood and CSF bank of ALS patients - we have expressed interest in participating in international industry sponsored clinical trial (decision pending) - although we have been a multidisciplinary team for years this has now been formalised with inclusion of several new members with specialist interest in genomics and sleep disorders - we will present our centre's activities at 6th national congress Dilemmas in neurology and initiate further regional cooperation - and last but definitely not the least - we have started validation and standardisation of the Croatian ECAS (Edinburgh Cognitive and Behavioural ALS screen) in collaboration with Prof. Abrahams' team from University of Edinburgh. Following this we intend to start the same process with DAS (Dimensional Apathy Scale) Although not as easily quantified we feel there has been a change involving not only our Centre activities but also our team's attitude from mechanistic to optimistic which is sometimes even more difficult to achieve.

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C12 Enhancing the efficacy of non-invasive ventilation for patients with amyotrophic lateral sclerosis

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Background. In patients with ALS, non-invasive ventilation (NIV) is the single most effective treatment available, with irrefutable benefits on both survival and quality of life. However, many patients do not gain the full benefit of the intervention and there is an absence of evidence to guide clinicians on how best to deliver NIV. Our review questions are: 1) What patient, carer, service and equipment factors are correlated with the optimal and suboptimal use of NIV in patients with ALS? 2) What are the best ways of initiating, and monitoring the efficacy of, NIV in these patients? Methods. MEDLINE, EMBASE, CENTRAL, CINAHL, AMED were searched from database inception to 1st October 2017, using keywords relating to population (ALS) and intervention (NIV), supplemented by reference list checks and key author contacts. We included quantitative and qualitative studies from the point at which a clinical decision to initiate NIV had been made, excluding those concerned with end of life care, optimal timing of NIV initiation, tracheostomy or other respiratory interventions. Risk of bias was assessed at full text level, with data extracted using a pre-piloted extraction form. Results. Of 4356 records screened, 69 quantitative and 13 qualitative studies met our inclusion criteria. We identified factors correlated with both the quantity of NIV use – patient acceptance, tolerance and adherence – and the quality of NIV – machine settings, interfaces and patient-ventilator interaction (e.g. leak, asynchrony). Solutions to causes of suboptimal ventilation are proposed. Comprising studies from Europe, North America, Asia and Australia, we have found substantial divergence in practice along several stages of the NIV pathway that may influence the efficacy of NIV. These include initiation settings, titration procedures, measures of ventilatory effectiveness and approaches to follow-up. Discussion. Preliminary findings suggest that, despite good adherence, some patients may be receiving inadequate ventilatory support and suboptimal benefit. This is the first phase of a wider project looking at how to optimise the use of NIV. Further phases include a mapping exercise across the UK and Europe to explore variation in clinical practice. We will combine evidence with clinician, patient and carer experience. Where there is evidential agreement, we will make best practice recommendations; where it conflicts, we will highlight areas for further research.

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C13 The radiological spectrum of motor-neuron diseases: A multimodal spinal cord study

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Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) type 3-4 are within the most common adult motor neuron diseases (MND). Despite great improvement in clinical and radiological description of ALS, only one study considered radiological characteristics of cervical spinal cord (SC) in SMA patients, while a comparison between the two diseases has never been done. Aim of the study: to describe SC MRI characteristics of adult type 3-4 SMA patients and to compare them with a corresponding ALS patient's cohort. Methods: 25 type 3-4 SMA, 25 ALS patients and 25 age-matched healthy controls (HC) underwent 3T cervical SC MRI acquisition. Anatomical imaging between C2 and C7 spinal levels included one sagittal, 3D, T2Đweighted, turbo spinĐecho sequence and a T2*-weighted multiĐecho gradient echo sequence providing high contrast for white (WM) and grey matter (GM). Diffusion tensor imaging (DTI) data were acquired using reduced field of view. MRI data were analyzed using the software "Spinal Cord Toolbox". Global, WM and GM cross-sectional area (CSA) at each vertebral level was computed as well as standard DTI parameters (FA, MD, AD, RD) for the cortico-spinal tracts (CST) and for the dorsal columns. Comparisons were made between SMA patients and HC and between SMA and ALS. Results: Both SMA and ALS patients presented a significant atrophy of the cervical SC between C2 and C7 when compared to HC ($p < 0,05$). No significant difference was found for global CSA between ALS and SMA patients, indicating similar entity of the atrophy. Significant atrophy of the GM ($p < 0,005$) was identified in SMA and ALS patients compared to HC, with ALS patients being significantly more atrophic than SMA. Significant atrophy of the WM was identified only in ALS patients compared to HC, but not in SMA patients. Any DTI alteration was found in the SMA group, while a significant reduction of FA in the CST and in the dorsal columns was found in the ALS cohort both compared to HC and to SMA patients. Conclusion: we describe specific GM atrophy of the cervical SC in SMA patients, which is not associated with WM atrophy nor with DTI alterations. These results suggest that SMA is a pure lower MND without involvement of the WM and clearly difference SMA from ALS, which is characterized by degeneration of both GM and WM and by FA reduction not only in the CST, but also in the dorsal columns, suggesting the presence of a multisystem involvement including also sensitive pathways.

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C14 Computational speech analysis as a tool for early detection of bulbar dysfunction in ALS patients

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INTRODUCTION: In amyotrophic lateral sclerosis (ALS) the initial symptoms of dysarthria are subtle and there is no objective method for assessing them, which can be difficult to early detection. We propose the computational analysis of speech as a method for the early detection of bulbar impairment in ALS patients. METHODS: An analysis of different speech acoustic variables using Praat software was performed. ALS patients with no apparent bulbar impairment, according to El Escorial criteria, were compared to healthy controls (H). Lingual strength, measured with IOPI system, was also included as alternative measure of bulbar involvement expression. RESULTS: 9 patients were evaluated and compared to 20 healthy volunteers. ALS patients compared to volunteer group presented a greater number of pauses (p=0.016), longer duration during the reading of a text (p = 0.005), as well as a lower verbal fluency (p <0.001). In addition, they presented a significant decrease in the lingual movement in the horizontal plane (p <0.001), with a trend towards a lingual anterior position (p=0.005) and inferior (p=0.003) during phoneme emission. The decrease in lingual strength was related (r=0.80) with a decrease in lingual movement (p=0.003). CONCLUSION: ALS patients without apparent bulbar dysfunction had an affectation of the lingual movements in the horizontal plane. The computational analysis of speech is a simple and objective tool that seems useful for an early detection of bulbar dysfunction in ALS and even allows to distinguish from other etiologies of dysarthria.

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C15 Prize4life ALS mobile analyzer: Measuring ALS progression

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ALS (Amyotrophic lateral sclerosis) is a progressive and incurable disease characterized by degeneration of the voluntary motor system. The disease was first described by Charcot in 1874, but became more famous in the 20th century, when it affected the baseball player Lou Gehrig and the cosmologist Stephen Hawking. ALS is a disease of the muscles and neurons: it is manifested in stiff muscles, muscle twitching, and gradually worsening weakness. This results in difficulties of movement, speech, swallowing, and eventually breath. The standard of care for assessing disease progression is the ALS Functional Rating Scale, Revised (ALSFRS-R), which is a pen-and-paper questionnaire aimed to evaluate patient daily living functioning. One of the major barriers for finding a cure for ALS is the enormous heterogenicity of the disease, which hampers the studying and testing of novel treatments, and the lack of an objective tool for monitoring disease progression. The ALS research community has long been interested in the ideas of developing methods to quantify disease progression in a way that improve the efficiency of clinical trials and minimize the physical burden that is placed on the patient who participates in those studies. Prize4Life is developing a smartphone-based application that monitors ALS patients in their natural environment to collect objective, ongoing, comprehensive daily-life status data. The application operates through the gold standard ALSFRS-R questionnaire along with simple tasks to accurately and continuously assess functional abilities in various motor functional areas: walking, arm lift, fine motor skills, breathing, and speech. Together, the application enables large-scale functional data collection that is expected to advance ALS research and to facilitate individually-tailored clinical care for ALS patients. Currently, Prize4Life continuing the research, development and clinical validation processes required to improve the app's functions and impact, including an improvement of the existing tasks, data collection methods, data analysis and user interface features. In parallel, we are working on research platform development and an option to create researchers clinicians access for specific patients.

1) Prize4life International, Ramat Hasharon, Israel.

C16 Clinical characteristics of amyotrophic lateral sclerosis patients from ALS center of the Republic of Srpska: case series and a review of literature

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Aim Disease progression and survival time of amyotrophic lateral sclerosis (ALS) patients is known to depend on several different factors and clinical presentation (phenotype) is one of them. Substantial epidemiological data from population-based registries suggests significant phenotype distinctions between different cohorts, probably due to environmental factors and genetic background. Our aim was to define prominent clinical features of sporadic ALS patients treated at ALS center of the Republic of Srpska, Bosnia and Herzegovina. Methods We performed a retrospective review of sporadic ALS patients treated at our ALS center, from January 2016 to January 2018. Data were collected for 30 patients, all of which were classified as probable or definitive ALS according to revised El Escorial criteria. Methods of descriptive statistics were used to summarize the data (standard deviation, median and T test). Results Male-to-female ratio was 2:1; out of 30 patients, twenty were male. Mean age at onset was 59,2±10,9 years. Disease onset spanned from 39-71 for male and 45-75 years of age for female patients, respectively. Majority of patients (16/30 or 53,33%) had a 12 months delay from the symptom onset to initial referral to physician. Analyzing the initial symptoms, we compared the spinal and bulbar form, and found 17 patients with spinal onset (57%) and 13 patients with bulbar onset (43%); patients with bulbar form were significantly older at the disease onset (64,9±8,8) compared to spinal form (54,8±10,8). Analyzing the distribution of fasciculations we found the head/tongue region to be most prevalent site (13 patients or 43,33%). Body weight loss was noted at 24 of patients (24/30 or 80%), most of them had a loss up to 10 kg of body weight (53,3%). Almost 90% of patients reported fatigue during regular daily activities and 63,33% of patients had a symptoms suggestive for depression. Pain was reported in 23 patients (76,66%), eleven of them had morning headaches and twelve pain in extremities. Conclusion The establishment of a cohort registry for ALS is able to determine clinical phenotypes and monitor the disease progression. Better ALS phenotypic characterization sets a background for further epidemiological studies, that could help improve our understanding of phenotype-genotype correlations, pathophysiology of the disease and possible contribute to new therapeutic approaches.

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C17 Smoking and ALS: Investigation of association followed by Mendelian randomisation analysis to assess causality

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Background: An association between smoking and amyotrophic lateral sclerosis (ALS) has been reported in observational studies, but whether smoking is causal of ALS remains unknown. In this study we investigated association between smoking and ALS in a UK population and used Mendelian randomization techniques to test causality. Methods: We first performed a case control study. Smoking status was collected using environmental questionnaires from people diagnosed with ALS between 2008 and 2013 at three centres in the UK, and from age, sex and geographically matched controls. We used logistic regression to assess whether there was a relationship between smoking and risk of ALS. We then used summary statistics from genome-wide association studies of smoking and ALS to perform two-sample Mendelian randomization analysis. We used inverse variance weighted estimation and MR-Egger regression to estimate the causal effect of smoking on ALS. Results: In the case control study there were 202 cases and 200 controls, all with complete data on smoking behaviour. Smoking at time of survey was associated with an increased risk of ALS (adjusted OR: 3.22 (95% CI 1.14-9.85), p = 0.032). No other smoking behaviors were associated with ALS. We found no evidence that smoking behavior was causal of ALS, but the analysis suffered from weak instrument bias. Conclusions: In line with other observational studies, we found that smoking is associated with increased risk of ALS. We were not able to confirm or refute a causal relationship using Mendelian randomization.

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C18 Genotypes and phenotypes of Amyotrophic Lateral Sclerosis in Mongolia

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C9orf72 hexanucleotide repeat expansions (HRE) account for the majority of amyotrophic lateral sclerosis (ALS) and a large proportion of frontotemporal dementia (FTD) cases in Western populations, but are rarely found in Asian populations. In contrast, Mutations in SOD1 are the most prevalent genetic cause in Asian populations. We aimed to genetically and clinically characterize ALS in 58 patients from Mongolia. Age of onset of disease (51.2±11.1 years) was substantially earlier in Mongolian patients compared to Caucasian populations, and the site of onset was frequently cervical (43.1%), rather than bulbar (31.0%), lumbosacral (24.1%) or thoracic (1.7%). The mean survival time of ALS was 60.0 (±6.7) months from the first paresis. We furthermore performed genetic testing on a total of 58 patients and 127 healthy individuals, including unaffected relatives of ALS patients, by Sanger sequencing, repeat-primed PCR and Southern blotting. Our analysis identified C9orf72 expansions with 1200-2400 repeats in 3 familial ALS index patients. Moreover, mutational analysis of SOD1 revealed 2 heterozygous and 1 homozygous D90A mutations in apparently sporadic cases of ALS. In contrast to reports from Western ALS populations, mutations in the FUS gene were not detected in a prospective cohort of early-onset (age at onset < 45) ALS patients from Mongolia (n=15). We furthermore conducted a comprehensive haplotype analysis on single-nucleotide polymorphisms (SNPs) surrounding the HRE in all three C9orf72 repeat expansion carriers and affected and unaffected family members, which suggests a population specific founder haplotype in Mongolia. Thus, we observed profound differences in the clinical manifestation of ALS in Mongolia when compared to Europe, and report a surprisingly high prevalence of C9orf72 mutations in Mongolia with a haplotype different from previously described C9orf72 HRE-associated haplotypes.

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C19 Euro-MOTOR: A multicentre population-based case-control study of dusts, gases and fumes as risk factors for ALS

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Objective: To investigate the exposure to dusts, gases and fumes based on full job histories using job-exposure matrices in a large multicentre, population-based case-control study with detailed information on possible confounders. Methods: Population-based ALS patients and controls were recruited from five registries in The Netherlands, Ireland and Italy. Demographics and data regarding educational level, smoking, alcohol habits and lifetime occupational history were obtained using a validated questionnaire. Using job-exposure matrices, we assessed occupational exposure to mineral dust, organic dust, gases and fumes in general, and more specifically to silica, asbestos, animal contact, endotoxin, polycyclic aromatic hydrocarbons and diesel motor exhaust. Multivariate logistic regression models adjusting for confounding factors were used to determine the association between these exposures and ALS risk. Results: We included 1,577 patients and 2,922 controls. Associations were positive for all nine occupational exposures (ORs ranging from 1.12 to 1.73), and significant for all but asbestos and animal contact. Subsequently adding an exposure to the model in the single exposure analysis revealed stable ORs for mineral dust and silica. We found similar results when patients with a C9orf72 mutation were excluded. Conclusion: In a large multicentre study using harmonized methodology to objectively quantify occupational exposure to dusts, gases and fumes, we found an association between ALS risk and exposure to mineral dust and silica, independent of the other occupational exposures studied.

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C20 Estimating future MND prevalence in the context of population change and putative new treatments, using a south London urban population

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Population size and demographic make-up is predicted to change over time and it is not clear how this will affect the incidence and prevalence of motor neuron disease in different age groups in the UK population. We used prospectively collected local clinic and registry data to generate incidence and prevalence estimates for motor neuron disease, based on a local population area with good case ascertainment (Lambeth, Southwark and Lewisham in south London). The overall age- and gender-adjusted incidence rate was 2.11 per 100,000 (95% C.I. 1.99 – 2.24), with a point prevalence of 5.01 per 100,000 (95% C.I. 3.60 – 6.96). Using the Office for National Statistics' UK population projections up to the year 2116, and assuming incidence rates remained stable, we estimated that population growth alone would result in an 83% increase in the number of new cases per year, from 1092 cases in 2010 to 2003 cases in 2116. We then used a range of established methods for estimating the future prevalence of MND in the UK, and modeled how these trends might be altered by putative new treatments that could prolong survival. This has important implications for healthcare planning and resourcing.

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C21 New insights into the pathophysiology of fasciculations in amyotrophic lateral sclerosis: An ultrasound study

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Introduction Fasciculations are characteristic of amyotrophic lateral sclerosis (ALS). The aim of this observational study is to describe the frequency of fasciculations in several muscle groups of ALS patients and to analyse their clinical and pathophysiological meaning. Methods ALS patients with a recent diagnosis (<90 days) were enrolled. Demographic and clinical data were prospectively collected and serum creatinine levels at diagnosis were recorded. Nineteen muscles representing the four anatomical regions were examined with ultrasound (US) in each patient. The number of fasciculations was recorded in each muscle and the muscle thickness and strength were measured in limb muscles. A subgroup of patients were electromyographically assessed. Results US was performed in 835 muscles of 44 patients and EMG was available in 263 muscles of 36 patients. US detected fasciculations more frequently than EMG. Fasciculations were widespread, especially in upper limbs onset patients and in the cervical region. Fasciculations inversely associated with ALSFR-R and body mass index (BMI) and directly with BMI loss and upper motor neuron (UMN) impairment. The association with BMI was not mediated by muscle mass loss. Fasciculations increased with the initial lower motor neuron (LMN) degeneration, reached their peak when the muscle became mildly to moderately weak, decreasing afterwards with increasing muscle weakness and atrophy. Conclusions Our study suggests a link between hyperexcitability, the UMN and LMN degeneration, fasciculations and BMI loss. Differences in fasciculations' rates within muscles could be explained by different stages of LMN impairment and between muscles by a variable degree of corticomotoneuronal input.

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C22 Stability and change: Needs of informal ALS caregivers across the caregiving course

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Objectives Caring for a partner or family member with a progressive neurological illness can be a source of burden and psychological distress. This study explores the expressed needs of informal caregivers across the ALS disease trajectory. Methods Primary informal caregivers of people with ALS attending the specialist National ALS/MND Centre at Beaumont Hospital, took part in a semi-structured home interview at three time points. They were asked to identify what would help them in their role of caregiver, and thematic analysis was used to identify themes from their responses. Results This was a largely female and spousal cohort of caregivers, living with the patient for whom they provide informal care. The majority of patients were was male (61%), and had spinal onset (68%). Themes developed included (1) External support and assistance (2) Patient-related factors (3) Psycho-emotional factors (4) Nothing (5) Cure-better. Conclusions These caregivers identified external support, psycho-emotional factors, patient resistance to outside services and a cure for ALS or at least respite from its progression, some responded that nothing would help. The findings illustrate the interrelatedness of external and internal resources. What caregivers consider helpful is comparatively consistent but the relative status of those needs changes over time and the course of caregiving.

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C23 Possible environmental factors associated with spatial clustering of ALS patients with C9orf72 mutations

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Background: The expansion of the hexanucleotide (GGGGCC) in the C9orf72 gene is the most important cause of ALS of genetic origin, accounting for approximately 40% of all familial cases of ALS and 7% of sporadic ALS, in which no apparent heritability has been reported. In fact, an interaction between genetic and environmental factors may be involved in its development. Our objectives are to find geographical clusters of ALS patients with the C9orf72 gene characterize the dominant phenotype in these clusters and determine environmental factors related to them. Methods: In our study, we use a population-based nested case-control study with 242 ALS patients, diagnosed between 2013 and 2016, 9.5% of which have the C9orf72 gen. The controls are ALS patients without the gene. The cases, ALS patients with the gene, are matched with controls by sex, year of birth and year of diagnosis. First, we use a mixed logistic regression, controlling for unobserved confounding. For it, we include in the regression random effects, which capture individual heterogeneity, and spatial and temporal dependence. In order to evaluate the existence of spatial clusters, we compute smoothed standardized incidence rates and exceedance probabilities. Second, we characterize the dominant phenotype in these clusters. Third, we include in the regression, as explanatory variables of interest, environmental variables (pesticides, air pollutants) and other covariates (sex, age, cognitive impairment, BMI, weight loss and a contextual deprivation index). Results: As preliminary results, in the group of carriers, 73.9% were women, while in the group of non-carriers, 45.6% were women. Regarding the phenotype, spinal onset was observed in 60.9% and bulbar in 39.1% of carrier patients, compared to 67.7% and 29.2%, respectively. 3.1% of non-carriers had a respiratory phenotype. The mean age at onset of symptoms was 57 years in carrier patients and 61.7 years in non-carriers. 52% of the patients had a family history of ALS and/or frontotemporal dementia (FTD), while only 6.6% had non-carriers. 70% of the carriers were diagnosed with FTD and 22.6% of the non-carriers. We observe certain spatial clusters, in some of them, the carrier dominate. Conclusion: Our preliminary results indicate that there are spatial clusters of ALS patients with the C9orf72, that the dominant phenotype of the carriers is the spinal, and that some environmental factors could have some role in the occurrence of such clusters.

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C24 Pathogenic biological routes common between sporadic amyotrophic lateral sclerosis (ALS) and ubiquitin frontotempoal lobar degeneration (FTLD-U)

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Background There is a common clinical spectrum between ALS and FTD (frontotemporal dementia), and a genetic and pathogenic overlap between both diseases has also been described. Based on neuropathology findings, sporadic ALS TDP 43 and FTLD-U are within the same disease spectrum (TDP-43 proteinopathies or tardopathies). For that, we consider appropriate to deeply map the neuroproteomes derived from spinal cord and non-motor cortex across the ALS/FTD spectrum. Material and Methods We carried out a differential proteome-wide analysis of both spinal cord and non-motor cortex area, derived from ALS subjects(n=8), ubiquitin-positive frontotemporal lobar dementia (FTLD-U) subjects(n=8) and neurological intact controls (n=8). Results At spinal cord level 20 differential proteins were shared between both neurological syndromes and at non-motor cortical level a proteome subset of 16 proteins was co-deregulated between ALS and FTD. Data mining of generated proteomic data emphasized the involvement of these proteins in cell survival, mitochondrial homeostasis, and neuron-specific functions. Conclusions ALS and FTLD-U share molecular and functional alterations at spinal and cortical level, although part of the proteostatic impairment is region and disease-specific.

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C25 Gene expression profile in frontal cortex in sporadic frontotemporal lobar degeneration-TDP

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Frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP) is the term used to designate different neurodegenerative processes presenting with behavioural-dysexecutive disorder, primary progressive aphasia and/or motor disorders including motor neuron disease. Neuron loss in the cerebral cortex; microvacuolation in the upper cortical layers accompanied by astrocytic gliosis; TDP-43-immunoreactive inclusions in the nucleus and/or cytoplasm of neurons and oligodendocytes, and in neuropil threads are the main neuropathological lesions. Some cases are sporadic (sFTLD-TDP) whereas other are genetic, often familial (fFTLD-TDP). The study of human brain tissue has been useful to unveil molecular alterations in FTLD-TDP. The present study was aimed at analyzing gene expression in frontal cortex area 8 in a series of sFTLD-TDP in parallel with controls in order to gain understanding about vulnerable pathways, which can explain pathogenic aspects of the disease. Assessment of 111 genes by RT-qPCR showed de-regulation of 81 genes linked to neurotransmission and synapses, neuronal architecture, cytoskeleton of axons and dendrites, vesicle trafficking, purines, mitochondria and energy metabolism in sFTLD-TDP. Western blotting studies disclosed down-regulation of several mitochondrial subunits encoded by genomic DNA and MT-CO1 encoded by the mitochondrial DNA. Mitochondrial ETC activity of complexes I, IV and V was decreased in sFTLD-TDP. These findings provide robust information about down-regulation of genes involved in vital biochemical pathways and in synaptic neurotransmission which may help to increase understanding about the biochemical substrates of clinical manifestations in sFTLD-TDP.

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C26 Capturing ALS: LCM-Seq for single-cell spatial transcriptomic profiling of human spinal motor neurons in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which somatic motor neurons in the cortex, brainstem and spinal cord progressively degenerate, leading to spasticity, muscle atrophy, and paralysis. The spinal motor neurons are often classified into three groups based on the type of muscle fiber they innervate; namely slow-twitch (S), fast-twitch fatigue-resistant (FR), and fast-twitch fast-fatigable (FF). The FF motor neurons are considered the most vulnerable in ALS, while S are comparably resilient. Although several functional characteristics of spinal motor neurons in man are long known, their transcriptional signatures and possibly greater diversity in health and ALS remain elusive. We study individual motor neuron somas in human post mortem spinal cord tissue using laser capture microscopy coupled with mRNA sequencing (LCM-Seq) in order to resolve their transcriptional diversity and further delineate subpopulations that are vulnerable and resilient to ALS. LCM-Seq enables both robust and sensitive detection of expressed genes. This technique permits the precise analysis of individual cells, while preserving the spatial information even in scarce and partly degraded human post mortem tissue. We present our preliminary data on the diversity of human spinal motor neurons at single cell resolution in control tissue and in donors succumbed to sporadic ALS. We anticipate that this analysis will further our understanding of the selective and differential vulnerability of spinal motor neurons and help delineate the underlying molecular mechanisms.

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C27 An ALS case with 38 (G4C2)-repeats in C9orf72 and sparse DPR and TDP-43 pathology

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The pathogenic (G4C2)-repeat length in most C9orf72 ALS patients is estimated to range from hundreds to thousands of repeat units, whereas controls show a repeat length of only 2 to 30 units. However, the shortest repeat length acting as the pathogenic threshold is still unknown. We present a case of a 64-years old man referred to the neuromuscular reference center of the University Hospitals of Leuven. The patient was diagnosed according to Awaji and revised El Escorial criteria with spinal onset probable ALS lab supported without cognitive/behavioral impairment and no family history of ALS or FTD. The patient's survival was only 28 months after symptom onset. Genotyping by triplet repeat primed PCR on peripheral blood DNA revealed a short repeat length of 38 (G4C2)-repeats in the C9orf72 gene. Dipeptide repeat protein (DPR) inclusions were evaluated by immunohistochemistry (IHC) in five distinct CNS regions including central cortex, hippocampus, frontal cortex, cerebellum and spinal cord. A semiquantitative grading system was used with inclusions rated as being absent, single, rare, moderate or numerous. For comparison, DPR inclusions in long repeat length C9orf72 cases (n=4) and controls (ALS, n=6 and nonALS, n=6) were evaluated as well. In the five CNS regions of the short repeat length case, only rare to moderate poly(GA) inclusions, rare poly(GP) inclusions and single poly(GR) inclusions were observed. This contrasts the pathology in the other C9orf72 cases where numerous poly(GA) inclusions, moderate to numerous poly(GP) inclusions and moderate poly(GR) inclusions were observed. By semiquantitative IHC analysis of the short repeat length case, more pTDP-43 pathology and loss of alpha motor neurons was observed in the spinal cord in comparison with the motor cortex. Scarce DPR pathology in a case with 30 (G4C2)-repeats has previously been reported (Gami et al, 2015). However, this case was clinically unaffected and was devoid of TDP-43 pathology, suggesting that a 30-unit repeat length is insufficient to trigger TDP-43 pathology. In contrast, our findings suggest that a 38-unit repeat length may be sufficient to trigger TDP-43 pathology in a disease-prone manner. This warrants further investigation to clarify the threshold of the (G4C2)-repeat length in C9orf72 for DPR and TDP-43 pathology and for the onset of ALS. L.D. is funded by the Research Foundation - Flanders (FWO). D.R.T. and P.V.D. received a grant by KUL-C1 internal funding.

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C28 Inhibition of Rho Kinase (ROCK) with Fasudil as disease-modifying treatment for ALS – a phase IIa clinical trial (ROCK-ALS)

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Background: An effective disease-modifying therapy for Amyotrophic Lateral Sclerosis (ALS) is still not available. The Rho kinase (ROCK) inhibitor Fasudil has demonstrated neuroprotective effects on motor neuron survival in preclinical studies, has been shown to enhance axonal regeneration and to modulate microglial function both in vitro and in vivo. For many years, Fasudil has been approved in Japan and China for the treatment of vasospasms following subarachnoid hemorrhage and has a favorable side effect profile. Objectives: To develop a safe and tolerable new disease-modifying therapy for ALS patients based on repurposing of Fasudil. Methods: ROCK-ALS is a phase IIa clinical trial investigating the safety, tolerability, and efficacy of Fasudil in ALS patients at an early stage of disease (EudraCT-Nr.: 2017-003676-31). Safety and tolerability will be primary objectives wheras efficacy is a secondary objective, which will be assessed by changes in ALSFRS-R, ALSAQ-5, vital capacity, and MUNIX as well as survival. In addition, the study will collect biomarker fluids (blood, cerebrospinal fluid, saliva and urine) for the correlation of surrogate parameters to clinical outcomes. The trial is expected to enroll a total of 120 patients with a probable or definite ALS (according to the revised El Escorial criteria) and a disease duration of at least 6 but not more than 18 months. A total of 15 centers in Germany, Switzerland and France will participate in this interventional, randomized study as part of an international consortium. An associated center in Poland will be recruiting patients in an independent, untreated biomarker cohort. In a three-arm design, patients will receive an intravenous dose of either 15 mg fasudil, 30 mg fasudil or placebo twice daily for a total of 20 days. Safety-relevant examinations will be carried out during the entire treatment period as well as in the follow-up period until day 180. Efficacy-relevant examinations will be performed before and immediately after the infusion therapy as well as on days 42, 84 and 180. Results: The ROCK-ALS trial is funded under the 2016 EU E-Rare program Clinical research for new therapeutic uses of already existing molecules (repurposing) in rare diseases (http://erare.eu/financed-projects/rock-als). Upon completion of regulatory approvals, ROCK-ALS plans to enroll first patients in 2018.

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C29 Leveraging crowdsourcing to advance novel therapeutic targets for ALS: The Teva CNS Target Identification Crowdsourcing Initiative

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Neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Parkinson's disease (PD), are becoming an increasing public health concern as the global population ages. Effective treatment options for these debilitating and fatal conditions are severely limited. Although there has been progress in recent years towards unraveling disease mechanisms, the underlying causes of these disorders are not well understood. Even when disease-causing mutations are identified, such as the ALS-causing mutations in SOD1 and C9orf72, it is still unclear how they lead to neuronal death, and ultimately, to the specific cognitive and/or motor deficits characteristic of each neurodegenerative condition. The key to discovering effective treatments lies in understanding the basic mechanism or pathophysiology of the condition, thus uncovering relevant pathways and targets for pharmaceutical intervention. Teva Pharmaceuticals, in partnership with the global pioneer in crowdsourced innovation, Innocentive, established the Teva CNS Target Identification Crowdsourcing Initiative. The goal of the initiative was to solicit creative proposals of potential new targets for the treatment of ALS and other central nervous system disorders, and to identify investigators with unique expertise around those targets. The challenge called on scientists to propose novel intracellular or extracellular targets pertinent to the pathophysiology of ALS, HD and PD, as well as migraine and pain, that may be modulated by small molecules or biologics. Proposals were sought that provided a strong rationale for the target's involvement in disease pathophysiology. The challenge leveraged the extensive network of hundreds of thousands of scientists connected through the online Innocentive platform, to reach a global community of scientists of diverse background and expertise. Solutions that met the technical requirements of the challenge were considered for cash awards and for a collaborative research project with Teva scientists. The program was launched in partnership with The ALS Association and The Huntington's Disease Society of America, who co-sponsored the challenge awards. Here we present a description of the challenge, the technical requirements for proposals of new targets, and an overview of the biological pathways represented in the ALS-related solutions.

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C30 The GPR17 receptor as a new potential pharmacological target to restore oligodendroglial dysfunction in amyotrophic lateral sclerosis

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Amiotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease due to loss of motor neurons (MN). Recently, it has been shown that, besides MNs, oligodendrocytes (OLs) also undergo very early death in ALS. As a consequence, oligodendrocyte precursor cells (OPCs) increased proliferation, but failed to replace degenerating oligodendrocytes. The differentiation capacity of OPCs is also impaired, resulting in the presence of immature and dysfunctional oligodendrocytes, unable to provide MN with trophic and metabolic support (Lee Y et al. 2012 Nature; Kang SH et al. 2013 Nat Neurosci). Restoring OL function and promoting OPC maturation thus emerge as interesting approaches to prevent MN degeneration. An important regulator of OPC differentiation and myelination is the membrane P2Y-like receptor GPR17, that is specifically expressed in OPCs in transition to pre-OLs, but not in mature cells. We have shown that prevention of the physiological GPR17 downregulation at late OPC stages through forced receptor over-expression results in delayed cell maturation (Fumagalli M et al. 2015 Glia). Accordingly, GPR17 over-expression has been described in several model of CNS degeneration characterized by remyelination failure (Fumagalli M et al. 2016 Neuropharmacol). On this basis, the aims of this work were to characterize GPR17 alterations in ALS pathology and to assess whether this receptor could be exploited as a new pharmacological target to restore OL dysfunction and MN loss. For the first aim, western blot and immunohistochemistry analysis were used to evaluate GPR17 expression in parallel to the mature OL marker MBP in the SOD1G93A mouse ALS model. Spinal cord (SC) lumbar tracts of SOD1G93A mice at different disease stages were compared with age-matched controls. We also examined the metabolic support function of oligodendrocytes by analyzing MCT1, a lactate transporter expressed by these cells. Data showed that in ALS mice the expression of MBP and MCT1 are reduced, whereas GPR17 expression was found to be significantly up-regulated. For the second aim, we isolated OPCs from SCs of Ctrl and SOD1G93A mice using MACS technology to assess in vitro their differentiation and proliferative abilities. Results indicated that OPCs from SOD1G93A showed a lower differentiation capability with respect to OPCs from Ctrl mice; no differences were detected in the proliferation rate. Experiments are ongoing to verify whether these alterations may be rescued by GPR17 ligand.

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C31 Targeting TGF-ß RII to treat Amyotrophic Lateral Sclerosis by a 3rd generation antisense oligonucleotide – in vivo safety and efficacy

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Objective: To test the in vivo safety and preliminary efficacy of a novel 3rd generation Antisense Oligonucleotide (ASO) against TGF-ßRII (BiAgil). Background: ALS patients exhibit an enhanced TGF-ß system activity critically mediating the imbalance of neurodegeneration and neuroregeneration. Aside from a genetic origin progressive neurodegeneration is due to a profound TGF- ß mediated pro-inflammatory and neurotoxic milieu, an arrested adult neurogenic niche activity, and an increased pro-fibrotic state. A TGF- ßRII specific LNA-Antisense Oligonucleotide was designed for therapeutic intervention and studied in vivo. Methods: We tested ASO tolerability by using a dose-escalation paradigm with increasing dose levels every single week (0.4 mg/animal to 20 mg/animal). One male and female Cynomolgus monkey each received an intrathecal ASO injection and physical/neurological parameters were investigated directly and following 4h after administration. In a second experiment ASO pharmacokinetics was evaluated for ASO tissue concentrations following 2 or 4 weeks after i.t. drug administration for two different ASO doses (low: 0.8 mg/animal; high: 4 mg/animal). CSF was taken to evaluate ASO half-life. Finally, in a third paradigm the ASO was injected repeatedly over a 13-week approach at 2 doses for long-time administration. Again, CNS tissue samples (spinal cord, brain) were collected to evaluated tissue distribution. Target regulation and the expression levels of the ligands and the most important downstream targets were determined within spinal cord, motor cortex, dentate gyrus, and the subventricular zone. Consequently, effects of an altered TGF-ß system activity on the adult neurogenic niche were followed by measuring neuronal stem cell markers. Results: The results of the current study indicate that BiAgil is well tolerated, stable and a potentially highly effective agent to regulate an overactive TGF-ß system. The target molecule is regulated within the CNS and spinal cord, initial results point to high functional activity. BiAgil may be a promising candidate to treat ALS progression. Supported by BMBF GO-Bio Grant

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C32 A placebo-controlled study to evaluate efficacy and safety of Clenbuterol in patients with Spinal and Bulbar Muscular Atrophy (SBMA)

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Spinal and bulbar muscular atrophy (SBMA) is an X-linked, adult-onset motor neuron disease caused by expansion of a polyglutamine-encoding trinucleotide repeat in the androgen receptor gene. At present, no treatment exists for SBMA. Clenbuterol is a beta2 agonist having anabolic effects on skeletal muscle. In addition, it activates the PI3K/Akt pathway, which is demonstrated to promote degradation of the abnormal AR protein. In a pilot trial in 20 patients with SBMA, clenbuterol, given at 0.04 mg/day for 12 months, was associated with a significant and sustained increase in walking distance covered in 6 minutes and in forced vital capacity. These findings require verification in a larger, controlled trial. Aim of the study: Primary objective of the study will be assessment of the efficacy of clenbuterol by evaluating changes in motor function according to validated functional parameters. Secondary objectives include assessment of the persistence of the effect after prolonged treatment and tolerability of the assigned treatment. Any potential clenbuterol-related muscle damage will be researched by muscle MRI. Methods: 90 SBMA patients will be enrolled in a multicenter, phase II, randomized, double-blind, placebo-controlled trial. Patients will be recruited by Italian secondary and tertiary neuromuscular centers. All eligible subjects will be randomized to receive either Clenbuterol 0,04 mg/d or placebo for 12 months. Efficacy of chronic treatment with Clenbuterol will be demonstrated by assessing its short-term impact on motor function as measured by performed tasks (6MWT, AMAT, fVC), and by functional scales. Anabolic effects will be also assessed by muscle Quantitative MRI. Patients will be evaluated every 3 months for a year. Potential side effects on the cardiovascular system will be evaluated by EKG at each time-point, while Echocardiogram and Holter-EKG will be performed at the beginning and at the end of the study. The tolerability of Clenbuterol will be also assessed by listing and counting all adverse events occurring during the trial and comparing active treatment and placebo. Attended results: Based on our previous data, the primary endpoint will be a 15% increase at 12 months at the 6MWT. In the absence of a drug with a documented effect on the progression of the disease, the trial aims at providing unequivocal evidence that Clenbuterol improves motor function to a significant extent compared to usual care (superiority trial).

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C33 Masitinib therapeutically targets sciatic nerve pathology associated with paralysis progression in an inherited ALS model

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Progressive spreading of skeletal muscle paralysis is a clinical feature of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease with an average survival of 2 to 5 years. Evidence in rodent models expressing ALS-linked SOD1 mutations indicate that specific glial and immune cells emerging after paralysis onset can accelerate disease progression. Masitinib inhibition of the tyrosine kinase receptors c-Kit and CSF-1R downregulates activated spinal cord glia cells as well as skeletal muscle mast cells, thus reducing the rate of post-paralysis motor neuron loss, NMJ denervation, and disease progression. However, it is presently unknown whether mast cells and neutrophils play a pathogenic role during spinal nerve degeneration in ALS. We have analyzed the progression of sciatic nerve pathology in SOD1G93A rats from the onset of hind limb paralysis until advanced paralysis over 15 days. We observed a previously unreported infiltration of degranulating mast cells into the endoneurium, the number of which sharply increased after paralysis onset and correlated with progression of nerve pathology. Notably, chymase-positive mast cells formed large heterotypic multicellular aggregates with elastase-positive neutrophils that were aligned along the sciatic nerve endoneurium in close contact with misfolded SOD1 and fragmented myelin ovoids. Mast cells also interacted with macrophages, which expressed the c-Kit receptor ligand stem cell factor (SCF), essential for mast cell differentiation. CSF-1R agonists, IL34 and CSF1, were expressed in GFAP-positive Schwann cells. Pharmacological inhibition of c-Kit and CSF-1R with oral masitinib (30 mg/kg/day) for 15 days from the start of paralysis onset, prevented the appearance of these mast cell/neutrophil aggregates and decreased the number of non-phagocytic macrophages. Remarkably, masitinib treatment also significantly decreased axonal pathology and demyelination, as compared to vehicle-treated rats. These findings provide additional evidence for mast cell and neutrophil-driven pathology of the sciatic nerve in ALS, effectors likely contributing to aggravate distal axonopathy. Moreover, the observation that this disease mechanism can be therapeutically targeted with masitinib provides further rationale for treating ALS with masitinib; its neuroprotective effect having now been reported in both the central and peripheral nervous systems.

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C34 Masitinib in the treatment of amyotrophic lateral sclerosis (ALS): Update on confirmatory phase 3 trial (AB14008)

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Background Masitinib, an oral tyrosine kinase inhibitor, appears unique among other ALS-developmental drugs, exerting neuroprotection by simultaneously targeting microglia, macrophage and mast cell activity, both in the central and peripheral nervous systems. Masitinib administered at 4.5 mg/kg/day as an add-on to riluzole has previously demonstrated a positive benefit-risk balance in ALS patients with an ALSFRS-R progression rate from disease-onset to baseline (ΔFS) of <1.1 points/month (study AB10015). Significant disease retardation was evident in terms of ΔALSFRS-R (slowed loss of function), survival-to-event analysis (delayed progression), ALSAQ-40 (slowed deterioration in quality-of-life), and FVC (slowed deterioration in respiratory function and surrogate strength test). The objective of study AB14008 (phase 3 randomized, controlled trial) is to confirm findings from study AB10015 while utilizing a dosing regimen designed to optimize the benefit-risk balance. Design An estimated 600 ALS patients assigned to 4 treatment-arms (1:1:1:1) will receive riluzole (50 mg b.i.d.) plus masitinib or placebo during 48 weeks. The study drug will be administered utilizing a dose escalating scheme to reach the assigned target dose; for example, starting dose of 3 mg/kg/day with switch to 4.5 mg/kg/day, and starting dose of 3 mg/kg/day with switch to 4.5 mg/kg/day then to 6 mg/kg/day, with each switch being subjected to a safety control. The study population comprises male and female patients in the age group of 18 to 81 years, with FVC ≥60% and diagnosed with laboratory supported, clinically probable or definite ALS (revised El Escorial criteria). Additionally, the rate of ALSFRS-R progression before randomization should be <1.1 point/month, baseline disease duration from diagnosis <18 months, and all 12 items of ALSFRS-R will have a non-zero score. Primary endpoint is absolute change in ALSFRS-R[W0–W48]. Secondary endpoints include survival-to-event analysis (defined as ALSFRS-R deterioration of >9 points from baseline or death), quality-of-life (ALSAQ40), Clinical Global Impression (CGI), muscle strength (HHD) and respiratory function (FVC). Safety analysis will include all patients administered at least one dose of study drug. Concluding remark A positive benefit-risk balance will confirm that masitinib is an important new therapeutic option in ALS.

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C35 Initiation of masitinib at a less severe stage of disease produces greater treatment-effect: Subgroup analyses from masitinib study AB10015

Jesus S. Mora (1)*, Angela Genge (2), (On behalf of the AB10015 Study Group), Colin D. Mansfield (3), Olivier Hermine (3,4)

Masitinib (MAS) has previously reported positive phase 3 findings in ALS. Here we present post-hoc analyses that explored whether patient (pt) susceptibility to MAS is influenced by baseline disease severity, as measured by the individual component scores of ALSFRS-R. Study AB10015 used a prospectively stratified design based on ALSFRS-R progression rate calculated from disease-onset to baseline (ΔFS). A dichotomizing cutoff at 1.1 points/month distinguished between Normal Progressor (NP, ΔFS<1.1) and Fast Progressor (FP, ΔFS≥1.1) pts. The assumption here is that heterogeneity in ALS disease aggressiveness reflects differing disease mechanisms, leading to divergent treatment susceptibility. This approach therefore defines a more homogeneous primary efficacy population (i.e. NP pts receiving oral MAS 4.5 mg/kg/day), while concurrently permitting evaluation of the more heterogeneous population (i.e. 'NP plus FP' pts receiving MAS 4.5 mg/kg/day). The primary outcome endpoint, decline in ALSFRS-R from baseline to week-48 (ΔALSFRS-R), showed benefit for MAS (n=99) over placebo (PBO) (n=102) with a between-group least-squares means difference (ΔLSM) of 3.39 (-9.24 vs -12.63), corresponding to a significant and clinically meaningful slowing in functional decline of 27% (P=0.016). Conversely, significance on ΔALSFRS-R was not reached for the 'NP plus FP' cohort (secondary analysis), with a ΔLSM of 2.09 in favor of MAS (P=0.12). Post-hoc analysis showed that initiation of MAS treatment at a less severe stage of disease produced greater treatment-effect for both ΔFS-stratified cohorts in terms of ΔALSFRS-R (as well as secondary endpoints of FVC, ALSAQ-40, and survival-to-event analysis). Notably, this minor adjustment in pt selection criteria revealed a significant benefit for MAS in the 'NP plus FP' cohort. For pts with a baseline score of ≥1 on each ALSFRS-R item, ΔALSFRS-R was -9.8 for MAS (n=92) vs -13.1 for PBO (n=105); a ΔLSM of 3.3 and 25% slower rate of decline (P=0.0266). For pts with ≥2 on each ALSFRS-R item, ΔALSFRS-R was -6.7 for MAS (n=48) vs -11.5 for PBO (n=57); a ΔLSM of 4.8 and 42% slower rate of decline (P=0.0152). Finally, for pts with ≥3 on each ALSFRS-R item, ΔALSFRS-R was -4.3 for MAS (n=20) vs -15.1 for PBO (n=27); a ΔLSM of 10.8 and 72% slower rate of decline (P=0.0064). Results indicate greater benefit is possible when initiating MAS at a less severe stage of disease, with a significant treatment-effect seen regardless of post-onset ΔFS.

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C36 Sensitivity analyses from the first phase 3 clinical study of masitinib (AB10015) in ALS demonstrate robustness of the positive primary analysis

Olivier Hermine* (1,2), Vincent Arnold (1), Colin D. Mansfield (1), Jesus S. Mora (3), Angela Genge (4) Genge (On behalf of the AB10015 Study Group)

Masitinib (MAS) has previously reported positive phase 3 (AB10015) findings in ALS, signaling that it could provide an important new treatment option. Here we present results from various sensitivity analyses on the primary endpoint. The primary efficacy population of study AB10015 was predefined as patients (pts) receiving MAS 4.5 mg/kg/day with a ALSFRS-R progression rate from disease-onset to baseline (ΔFS) of <1.1 points/month. The primary endpoint, decline in ALSFRS-R from baseline to week-48 (ΔALSFRS-R), showed significant benefit for MAS (n=99) over placebo (n=102) with a between-group least-squares means difference (ΔLSM) of 3.39 (-9.24 vs -12.63); 95%CI 0.65–6.13, P=0.016. Missing data were imputed via last observation carried forward (LOCF) methodology for those pts discontinuing because of toxicity or lack of efficacy before week 48. In total, six predefined sensitivity analyses were conducted on the primary analysis, including two full analysis dataset (non-LOCF) imputation methods and four variations on LOCF via censoring on reason for discontinuation. All results from these sensitivity analyses were consistent with the significant outcome of the primary analysis, corroborating the robustness of this finding. Considering the most pessimistic full analysis dataset (imputation with penalty), which estimates progression for similarly clustered pts then imputes missing values using this average trend, ΔALSFRS-R for MAS (n=104) was -11.4 versus -14.4 for placebo (n=111); corresponding to a ΔLSM of 3.0 and significant 26% slowing in rate of decline (P=0.018). This result was further verified via tipping point analysis, a form of stress testing that assesses how large departures from the 'Missing at Random' assumption must be to overturn conclusions from the primary analysis. The tipping point was achieved with a penalty of 76% (i.e. as if 76% of discontinued MAS pts were equivalent to placebo). Because this is an improbable scenario, we can say that the analysis is robust. Additionally, a multiple imputation method was performed post-hoc using a monotone regression model (with initial conditioning by MCMC imputation). Results showed a significant slowing in decline of ALSFRS-R for MAS (n=105) versus placebo (n=113) by 21%, with ΔLSM of 2.8 (-11 vs -13.8); (P=0.0454). Taken together, these positive results from multiple/single imputation sensitivity analyses corroborate the robustness of study AB10015 primary endpoint data.

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C37 People living with ALS and their caregivers' input into drug development

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There is a rising call for rigorous patient input into key areas of regulatory consideration, such as clinically meaningful outcomes and benefit/risk calculations, as well as an emerging focus on patient engagement in determinations of value for health plan coverage and payment. Together, these developments offer a critical opportunity to use established methods to ensure that patient input is appropriately and adequately integrated into drug development. To respond to this opportunity and need, The ALS Association is developing a patient and caregiver-driven initiative – ALS PREFER – a cross-sector collaboration to build a robust, pre-competitive solution for patient preference studies in ALS with funding support from industry, non-profit organizations and other stakeholders. The initiative will be conducted in collaboration with the broader patient advocacy community, industry stakeholders, academic and clinical partners, and government representatives. It will draw on the community momentum behind the draft ALS Drug Development Guidance which led to the FDAâ€™s release in February 2018 of a draft guidance for public comment. The Guidance project engaged nearly 40 people with ALS and their caregivers, more than 10 ALS advocacy organizations, 45 of the world's leading ALS researchers and clinicians from 30 different institutions, 15 representatives from 9 biopharmaceutical companies, and 5 government representatives from the three centers at the National Institutes of Health and the Centers for Disease Control and Prevention. IMPACT ALS, a US focused effort, is the first of many initiatives that will become a part of ALS PREFER. A similar effort is planned in Europe (refer to abstract). Rigorous patient and caregiver input is warranted to guide drug development, but existing information regarding the burden of ALS is limited and gleaned only from small or geographically restricted studies. IMPACT-ALS is a survey initiative to expand the available data for patient and caregiver perspectives in order to guide drug development. Results from the survey will be presented at the meeting.

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C38 Long-term Outcome of Filgrastim (G-CSF) in ALS Patients

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Objective: To investigate the outcome of long-term G-CSF treatment in ALS patients, and to unveil biomarkers potentially reflecting prognosis and mode of action of G-CSF. Background: Previous efforts investigating the potential role of G-CSF in ALS patients have not provided convincing evidence of efficacy, possibly due to short treatment duration, or limited dosage in this potentially regenerative approach. Methods: 37 (36 evaluable) definite ALS patients (mean age 52 yrs, 25m/11f) were included with informed written consent on an outpatient and named patient basis from 2010 to present, with a mean dose of 351 Mio IU G-CSF/mo, and a median treatment duration of 13.7 mo. Results were compared to the PRO-ACT database and analyzed for biomarkers (monthly stem cell-, cytokine-, chemokine-markers; MR-DTI fractional anisotropy every 3 mo). An individual survival prediction model was constructed based upon the PRO-ACT database. Results: Safety, feasibility and tolerance were excellent. Adjusted (age, sex, baseline ALS-FRS-R, riluzole, site of onset, time-lapse from diagnosis to therapy) multi parameter survival analysis revealed a significant benefit in favor of G-CSF compared to PRO-ACT. A matched-pair analysis (25 PRO-ACT pts vs 1 G-CSF pat, bootstrapping, leave-one-out procedure x 10^4) resulted in a significant difference of survival with a median of 367 (PRO-ACT) vs. 609 (G-CSF) days post treatment start. Observed survival correlated significantly to estimated survival as assessed by the individual survival prediction model within PRO-ACT ($p<0,0001$). Differences between observed to PRO-ACT-predicted survival times were significantly in favor for G-CSF. Biomarkers correlated with prognosis and with G-CSF response were monitored in individual patients. Individual response was significantly associated with patients' hematopoietic stem cell mobilization. Although there was a significant general benefit of G-CSF in ALS, a highly responsive subgroup of ALS patients with a distinct biomarker profile could be delineated. Conclusions: Long-time G-CSF therapy in ALS patients seems extremely encouraging. Biomarkers that supervise this immune modulatory and stem cell activating therapy need further validation. It will help to identify a clear cut highly responsive subpopulation, who might have a substantial benefit from this safe outpatient therapy. Supported in part by BMBF-GO-Bio Grant

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Poster Session 2: Thursday 21st June, 17:45 - 19:15

Entrance Hall:

B01 Discovery and characterisation of a novel genetic variant of amyotrophic lateral sclerosis

Tobias Moll*, Dr Johnathan Cooper-Knock (1), Dr Alexander Beer (1), Dr Henry Robbins (1), Dr Adrian Higginbottom (1), Dr Guillaume Hautbergue (1), Dr Lydia Castelli (1), Dr Tennore Ramesh (1), Dr Janine Kirby (1), Prof Dame Pamela Shaw (1)

Introduction: Current understanding of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is based primarily on the study of genetic subtypes. Discovering new genetic causes of disease offers a powerful platform for building disease models and identifying therapeutic targets, particularly when the new gene occurs in a distinct functional pathway. Methodology: Whole exome sequencing was used to identify a causal genetic variant in related individuals with autosomal dominant ALS. This was followed by targeted sequencing of candidate genes in a cohort of 103 familial and young sporadic ALS (sALS) cases. We used immunocytochemistry (ICC) to confirm intracellular localisation of wild-type (WT) and mutant forms of the corresponding protein. The functional impact of these mutations in neuronal (N2A) and non-neuronal (HEK293) cells was assessed by MTT and lactate dehydrogenase assays. Knockdown of the gene in zebrafish embryos was performed using morpholino oligonucleotides. Results: Only one genetic variant was present in both family members with autosomal dominant ALS, absent from controls ((n=220 local, n=60,000 in ExAC), and found in additional ALS cases (n=4). In advance of first publication, the gene shall be referred to as 'X'. No patients with a mutation in gene X had a coexisting mutation in a known ALS gene, and disease was within the spectrum of sALS. 5 patients with p.R92C mutations suffered more aggressive disease with mean survival of 13 months, but the single patient with a p.G78W mutation lived >5 years. ICC showed localisation of protein 'X' to the Golgi network in N2A and HEK293 cells. When compared to WT protein we have shown that mutant forms are cytotoxic and reduce cellular metabolism, consistent with a gain-of-function toxicity. The p.R92C mutation was more toxic than p.G78W in all assays which is in-line with observed clinical severity. Knockdown of gene 'X' produced a motor phenotype in zebrafish embryos at 5 days post-fertilisation, suggesting gene 'X' is integral to motor system function. Conclusion: Protein 'X' is expressed in neurons and thought to localise to

the Golgi network. Functionally protein 'X' is poorly characterised but it contains a glycosyltransferase domain. Glycosyltransferase activity is associated with ganglioside synthesis which is disrupted in ALS. This is the first time a gene in this pathway has been identified in ALS patients, offering an exciting opportunity for therapeutic intervention.

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B02 Characterisation of a cohort of adult onset Middle Eastern ALS cases for mutations in known ALS genes

Nada Al-Ahmady* 1,2, Martina de Majo 1, Simon Topp 1, Chun-Hao Wong 1, Christopher Shaw 1, Marc Gotkine 3, Bradley Smith 1

Introduction Amyotrophic lateral sclerosis is a fatal neurodegenerative disease caused by genetic mutations transmitted with a clear familial Mendelian inheritance in ~10% of cases (fALS) or alternatively by lower penetrant mutations or complex susceptibility alleles presenting in a sporadic manner (sALS).. In the last two decades, disease causing mutations in ~35 ALS genes have been identified, accounting for ~20% of total ALS cases. We have recently conducted exome sequencing in a cohort of 110 Middle Eastern ALS cases (Arab and Jewish ethnicity) with 8% of cases documented to be consanguineous. The cohort is predominantly composed of classical ALS cases with adult disease onset, 9% of whom have a family history of disease. We aim to identify recessive genes or alleles in this patient set that also might be enriched in Caucasian fALS and sALS cases. However, we are also highly interested in the genetic architecture of this cohort in terms of mutations in known ALS genes and data on this aspect will be presented here. Methods All cases were prescreened for the intronic GGGGCC hexanucleotide C9ORF72 expansion prior to exome capture. Remaining cases were captured using the Nimblegen V3 probe set and individual exome libraries pooled (4 per lane) and run on an Illumina Hiseq3000 with raw data analysed for novel or exceedingly rare variants using a custom built in-house pipeline. Mutations were confirmed by Sanger sequencing. Results Eight individuals harbored mutations in the major dominant ALS genes. These comprised of four heterozygous C9ORF72 expansions, a homozygous D90A, a heterozygous p.R115C and a heterozygous p.L145F SOD1 mutation, and a heterozygous p.514_515del inframe FUS deletion). Of note, our cohort was abundant in OPTN mutations with one Palestinian family harbouring a novel p.S174X mutation that segregated with disease in a recessive manner and was absent in 1000 ethnically matched controls, and a homozygous novel essential splice site mutation which was identified in two unrelated Arab sALS cases, indicating a possible common founder. Conclusions This study shows that C9orf72 and SOD1 are amongst the most frequently mutated genes within our Middle Eastern cohort, in line with results seen in most western populations. The high incidence of homozygous OPTN loss-of-function mutations (3% of the cohort) mirrors that found by Goldstein et al in Israeli ALS cases of Moroccan and Ashkenazi Jewish ancestry (Neurology 86:5, 2016).

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B03 Genetic analysis of a French cohort of patients with sporadic amyotrophic lateral sclerosis (SALS)

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INTRODUCTION. Amyotrophic Lateral Sclerosis (ALS) is an adult neurodegenerative disease characterized by the progressive death of motor neurons. More than 30 genes have been implicated in the familial forms of the disease, but these forms represent only 5-10 % of all ALS cases, the majority are sporadic cases (SALS). In this study we analyzed 31 genes in a French cohort of SALS patients. The panel of genes was chosen in consultation with the French network of ALS Centers and FILSLAN (French rare diseases Healthcare Network: Amyotrophic lateral sclerosis and rare motor neuron diseases). METHODS. The hexanucleotide repeat expansion (HRE) in C9ORF72 gene was studied by repeat PCR/Genescan. The other 30 genes were analyzed by Next Generation Sequencing (NGS) and Sanger sequencing (3130xl Applied). For NGS, the libraries generated (Haloplex panel, Agilent) were qualified, quantified, and sequenced on MiSeq (Illumina) and analyzed by a pipeline developed in Tours (INSERM U1253-Center SLA). RESULTS. The cohort consisted in 355 SLAS patients with 56% men and 44% women. The average age of onset of the first symptoms was 59 years old. The C9ORF72 gene was mutated in 7.6% of patients; 20% of them reported the presence of behavioral disorders in their families (frontotemporal dementia, Alzheimer's). We next used NGS and Sanger sequencing to analyzed SOD1, TARDP and FUS and found heterozygous mutations in these genes in respectively 1.2%, 0.8% and 0.5% of patients. Novel mutations were identified such as for example p.Y526C in FUS (25-year-old woman; last amino acid) and p.N259S in TDP-43 (first mutation in the RRM2 domain). Several heterozygous missense mutations were also described in others genes, some were novel mutations. Their pathogenicity is being analyzed particularly in patients with mutations in two different genes. DISCUSSION. It is important to obtain more information about genetic of SLAS as this will help for molecular diagnosis and better understanding of the pathophysiology of the sporadic forms of the disease. In our study we described new mutations, some interesting for structure-function studies. This work also reinforces the interest of NGS for the study of a disease where oligogenism or polygenism could play an important role.

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B04 Next Generation Sequencing in familial ALS and/or FTD Spanish patients

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Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia form a clinical, pathological and genetic continuum. The aim of this study is to evaluate the suitability of performing a more complete genetic study sharing between familial ALS or FTD patients and ALS patients with FTD comorbidity. Methods: The number of ALS samples in "i+12" biobank is around 950 taking into account familial and sporadic cases, and ALS-FTD. The genetic studies performed in every sample were SOD1 and C9orf72 expansion. 39 patients have been selected in this study with a family history and who had not been previously characterized. The final study consisted on a customized panel (NGS) including 48 genes previously described as ALS or FTD causing or risk genes, and some of them related to Alzheimer disease, Charcot Marie-Tooth, Hereditary Spastic Paraplegia or Ataxia. Results: We identified 8 patients (20.5%) carriers of a variant previously published as pathogenic and causing the disease, with the TARDBP gene being the most frequent (n=5, 12.5%). There were also carriers of a pathogenic variant in TBK1, SQSTM1 and SPG7. Moreover, we found another 10 probably pathogenic variants in genes such as TBK1, MAPT, KIF5a, TUBA4A, PSEN1, FLNC (2 variants), DAO, DCTN1 and EPB41L1. Discussion: Our results suggest the existence of a wide range of genes involved in familial ALS, albeit to a lesser extent. In this way, it might be advisable to change the currently more generalized method (Sanger sequencing of the most common genes) to carry out a massive sequencing study aimed at a selection of genes that allows the genetic characterization of an extensive percentage of familial ALS and familial FTD. Keywords: gene panel, NGS, ALS, FTD, Acknowledgements This work was supported by grants PI14/00088 from the Instituto de Salud Carlos III (ISCIII), the support of the Spanish Foundation for the development of ALS research (FUNDELA) and Plataforma de afectados de ELA. References: 1. Al-Chalabi, A. et al., 2012. The genetics and neuropathology of amyotrophic lateral sclerosis. Acta Neuropathologica, 124(3), pp.339–352. 2. Renton, A.E., Chiò, A. & Traynor, B.J., 2014. State of play in amyotrophic lateral sclerosis genetics. Nature, 17(1), pp.17–23. 3. Robberecht, W. & Philips, T., 2013. The changing scene of amyotrophic lateral sclerosis. Nature neuroscience, pp.248–264.

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B05 Another pleiotropic gene, KIF5A, implicated in Turkish families with ALS and HSP

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Autosomal dominant missense mutations in the N-terminal motor domains of Kinesin Family Member 5A (KIF5A) have been commonly associated with spastic paraplegia 10 (SPG10) or Charcot-Marie-Tooth Type 2 (CMT2). Phenotypic heterogeneity is prominent among cases ranging from pure to complex forms of hereditary spastic paraplegia (HSP) with additional features like axonal sensorimotor peripheral neuropathy or cognitive impairment. Furthermore, heterozygous de novo frameshift mutations, located in the C-terminal region of KIF5A, are reported as the cause of a severe developmental syndrome called neonatal intractable myoclonus. Recently, a hotspot region in the C-terminal of KIF5A was shown to be responsible for classical amyotrophic lateral sclerosis (ALS), a more severe phenotype, by causing aberrant pre-mRNA splicing and thus, haploinsufficiency. In this study, we present Turkish ALS and HSP families with KIF5A mutations. Whole exome sequencing was used to identify the causative genes and Sanger sequencing for their validation. Two different heterozygous non-synonymous missense variations (c.3005A>G, p.(Asp1002Gly) and c.2927C>T, p.(Thr976Ile)) (NM_004984.2) located in the C-terminal of the KIF5A gene were identified in three individuals from two families with adult-onset ALS. The p.(Asp1002Gly) variation is a novel change, whereas the p.(Thr976Ile) (rs139801016) (ExAC MAF: 0.0002) variant is also observed in a second patient in our cohort with juvenile-ALS and a FUS mutation. Considering the loss-of-function nature of the KIF5A splice site mutations so far linked to ALS, the segregation analysis in extended family members is ongoing, in order to asses the pathogenicity of the above variants. Along this line, a heterozygous c.1379G>A, p.(Arg460Gln) mutation located further N-terminal of the KIF5A gene was observed in a Turkish family with an initial clinical diagnosis of ataxia accompanied by spasticity in lower extremities and mild cognitive deficit; the family harbours several affected individuals. Overlapping features are common among HSP, ALS and CMT, thus detailed clinical information, e.g. deep phenotyping along with molecular analysis, become extremely crucial to avoid false diagnoses and determine the extent of pathogenicity. This study aims to establish through a tight collaboration with expert clinicians, an accurate differential diagnosis to distinguish between these disorders with often similar clinical characteristics.

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B06 Dissecting the role of two novel ALS risk genes NEK1 and C21orf2

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Project MinE, an international whole-genome sequencing initiative, has identified two novel ALS 'risk genes' NEK1 and C21orf2. To date only little is known about function of C21orf2 and neither of the two proteins has been studied in neurons. Our project is aiming to elucidate function of these two proteins in nervous system and their relationship to ALS pathology. According to available expression data (e.g. http://biogps.org) both proteins show ubiquitous expression throughout various tissues probably due to their connection to primary cilia that are present in most mammalian cell types. We have performed detailed in situ hybridization and immunochemistry studies to closer analyze expression of these two proteins in the mouse nervous system. Both proteins show prominent expression in cortex, Purkinje cells of the cerebellum, DRGs and in motor neurons of spinal cord, i.e. in motor circuits' neurons. NEK1 and C21orf2 have been already shown to interact in vitro. We have mapped the interaction site between the two proteins using co-IP experiments with deletion mutants designed based on the known domain structure of NEK1 and in the case of C21orf2 its domain structure predicted by online tools (e.g. http://raptorx.uchicago.edu). The N-terminal part of C21orf2 and the C-terminal part of NEK1 are required for the interaction of these two proteins. Using online bioinformatics tools, we further identified a NLS signal in the N-terminal part of C21orf2 and a NES signal in its C-terminal part. Indeed, a C21orf2 construct lacking the C-terminal part localizes into nucleus when expressed in N2a cells. To analyze the relationship of NEK1 and C21orf2 to ALS we have created a set of mutant variants for each protein based on Project MinE data and mutations connected to ciliopathies. It is already obvious that some of these mutations have impact on the interaction between NEK1 and C21orf2 and some mutations can be linked to both ALS and ciliopathies. We are currently examining impact of these mutations in neuronal cells using mutant variant constructs. Furthermore, to substantiate the data we are creating iPSC-derived human motor neurons bearing some of these mutations using donor tissue samples and/or the CRISPR/Cas9 DNA editing system. Altogether, we are aiming to increase our understanding of the pathology of ALS and to create relevant ALS model(s) for performing screens focused on identifying molecules for therapeutic applications.

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B07 CAG Intermediate-repeats expansion in ATXN2 associated with increase of risk in ALS

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Amyotrophic lateral sclerosis (ALS), usually known as motor neuron disease, is a fatal neurodegenerative disorder characterized by a rapid-onset loss of upper and lower motor neurons. ALS has no cure, and its underlying cause is unknown in the majority of cases, although a strong genetic component is known to play a role. The gene ATXN2 normally has a repeat structure of around 22-23 triplets encoding for glutamine (CAG) within the reading frame of the gene encoding the ataxin two protein. Studies have shown that harbouring more than 40 repeats causes spinocerebellar ataxia type 2 (SCA2). Recently, it was discovered that intermediate-length repeat expansions (27-33 repeats) in ATXN2 are significantly associated with the risk of developing ALS. The aim of this project is to genotype the ATXN2 gene in a cohort of controls and patients from the Irish ALS bank in order to assess the association between this genotype and ALS. For ALS patients, the ATXN2 gene had a higher incidence of repeat expansions (between 27-32) than the control subjects. With regards to the intermediate repeat length expansion (between 24-34) in the ATXN2 gene, the ALS patients and the controls showed a significant repeat count variation (P= 6.027 x 10-3), with an increase of risk up to 11.34 times (CI 95% 4.10-31.39). This study further exemplifies the correlation between this gene and ALS in the Irish population, contributing to the research of causative genes for this devastating disease. Current work is extending our research into assessing the length of repeat expansions in other ataxia-associated genes, including ATXN1.

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B08 ALSscan: A framework for the analysis and visualisation of DNA NGS data of ALS patients

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Amyotrophic lateral sclerosis (ALS) is a progressive and uniformly fatal neurodegenerative disease. A plethora of genetic factors have been identified that drive the degeneration of motor neurons in ALS, increase susceptibility to the disease or influence the rate of its progression. DNA Next Generation Sequencing (NGS) data is a commonly applied approach for studying the genetic basis of this disease and underpins the aspirations of precision medicine including genetic diagnostic testing. However, there are significant challenges when dealing with NGS data. A huge number of bioinformatics tools exist and it is therefore challenging to design an analysis pipeline; NGS analysis is computationally intensive, requiring expensive infrastructure which can be problematic given that many medical and research centres do not have adequate high performance computing facilities and the use of cloud computing facilities is not always possible due to privacy and ownership issues. Moreover, the medical interpretation of the genetic findings is not trivial. We have therefore developed ALSscan, a complete framework for the analysis, annotation, and visualisation of DNA NGS data designed for ALS genetics. The framework consists of i) a fast and efficient bioinformatics pipeline that allows for the analysis of DNA sequencing data on a midrange computer. Focusing on the ALS related genes, the pipeline can analyse 40x WGS data in about 8 hours and whole-exome-sequencing (WES) data in 1 hour using a machine with 4 CPUs (4 cores) and 16 Gb of RAM, detecting SNVs, small indels, and a wide range of structural variants including repeat expansions. ii) An ALS genetics based annotation that makes use of public databases, e.g. ALSoD, ClinVar, CADD and GTR, as well as a manual literature review to perform gene and single variant prioritisation and favour the interpretation of the results iii) and report and visualisation utilities to make the framework suitable to a wider audience. ALSscan is available on GitHub (https://github.com/KHP-Informatics/DNAscan) as part of the DNAscan software suite. The repository provides detailed instructions for tool usage and installation. A bash script for an automated installation of the required dependencies is also provided as well as Docker and Singularity images for a fast and reliable deployment.

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B09 Estimating copy number of SMN1 and SMN2 gene using whole genome sequencing ALS survival

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Background Amyotrophic lateral sclerosis (ALS) usually leads to death within 3 to 5 years, but a high variability in patient survival has been observed, with 5% of the patients surviving more than 10 years [1]. To date, several clinical factors have been associated with patient survival, e.g. gender, age at onset, site of onset, presence of frontotemporal dementia, … [2]. Additionally, also genetic variants, like the C9orf72 repeat expansions, have been shown to associate with survival. Recent studies showed an association between the copy number variations (CNVs) of the SMN-genes may modulate the clinical severity ALS [3]. Objectives To identify CNVs in the SMN genes using whole genome sequencing (WGS) data and correlate these will ALS phenotypes like survival, onset and progression rate. Methods We used WGS data from 2,229 Belgian and Dutch ALS patients, sequenced as part of Project MinE. To estimate CNVs of SMN1 and SMN2 who are almost identical, we looked at three point mutations than can distinguish between SMN1 and SMN2 located on chromosome 5 at position 70,247,724, 70,247,773 and 70,247,921 for SMN1 and 69,372,304, 69,372,353 and 69,372,501 for SMN2 (genome build hg19) as suggested by Larson et al [4]. Using the read depth at these positions as surrogate for the average coverage of the SMN genes and 20 housekeeping genes with a stable CNV, the effective CNV of SMN1 and SMN2 will be estimated. Cox-regression was applied on the CNV of SMN correcting for age at onset, site of onset, gender, sequencing technology and the first 10 PCA's for population stratification. Results Based on the CNV estimates from WGS data we observed a reduced numbers of copies of SMN2 in ALS patients compared to controls. A difference in survival or onset was however not observed. Other populations within project MinE will be investigated to see if the associations are population specific. References 1. Pupillo E, Messina P, Logroscino G, et al. Ann Neurol. 2014; 75:287-297. 2. Chio A, Calvo A, Dossena M, et al. ALS. 2009; 10 205-209. 3. Butchbach MER, Front Mol Biosci. 2016 4. Larson JL, Silver AJ, Chan D, et al. BMC Medical Genetics. 2015; 16:100. Acknowledgements Samples were sequenced as part of the MinE project. Belgian samples were collected at UZ Leuven and Dutch samples at UMC Utrecht.

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B10 Determining the risk of ALS in relatives of patients with ALS: A study of re-categorisation rates from "sporadic ALS" to "familial ALS"

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Introduction: ALS may be distinguished into familial ALS (FALS) or sporadic ALS (SALS) based on the respective presence or absence of family history of ALS. There are numerous reasons why some FALS individuals may be misclassified as sporadic ALS including incomplete ascertainment of extended kindreds and small family sizes. Using standardised classification criteria for FALS applied to the Irish population based ALS register over a 23-year period, we sought to determine the frequency of re-categorization from SALS to FALS in an Irish ALS population. Methods: 269 individuals with familial ALS were grouped by whether they were identified as FALS at time of diagnosis or were classified initially as SALS. Annual rates of re-categorization were calculated by dividing the number of re-categorized FALS individuals by the number of individuals diagnosed with ALS annually. Results: 100 individuals, (65% male, 58% spinal onset), initially classified as SALS, were later re-categorized as FALS, with an overall mean rate of re-categorization from SALS to FALS of 3% (95% CI 2.6-3.8) per annum. Full clinical details were available on both the initial proband and second affected relative in 43 cases. In these families, the second affected relative was diagnosed a mean of 7.6 years (95% CI 5.9-9.2) after the first affected relative. In 44% of cases, the second relative was a first-degree relative of the first. Discussion: Differences in re-categorization rates from SALS to FALS in our study compared to that of a London based population (3% v 1.2% respectively) may be explained in part by differences in classification criteria used. Conclusions: Our findings show that the presence of Mendelian inherited genes in apparently sporadic patients is likely a function of mis-classification based on incomplete family history information.

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B11 Deciphering the respective contribution of macrophages and microglia to human motor neuron degeneration in ALS

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In ALS, while initiation of the disease seems to be intrinsic to motor neurons, its progression seems to involve non-neuronal cells in the environment of the motor neurons. Since spinal motor neurons have their soma in the central nervous system (CNS) while their axons lie at the periphery in contact to muscles, it involves two distinct micro environments: in the CNS, motor neuron soma is surrounded by microglial cells, the macrophages of the CNS, while at the periphery the axon is in surrounded by macrophages. We therefore want to analyze the respective contribution of macrophages and microglial cells to motor neuron degeneration using new cellular models derived from induced pluripotent stem cells (iPSc). iPSc clones from familial and sporadic ALS patients are already available in the lab and protocols were established to generate pure cultures of spinal motor neurons, macrophages and more recently microglial cells. Microglial cells and macrophages specificities are currently being characterized with specific markers using flow cytometry analyses, immunofluorescence labeling and qRT-PCR. To further study the specific physiological interactions between microglial cells and motor neuron soma, or macrophages and motor neuron axons, microfluidic devices will be used. This technology consists of two cell culture chambers separated by asymmetrical micro channels imposing the axonal growth in a single direction. We already successfully cultured iPSc-derived motor neurons in such microfluidic devices, and we observed the unidirectional growth of axons and the physical separation of motor neurons somas and axons. Our future goal is to study the specific toxicity mediated by macrophages or microglial cells towards ALS motor neuron and to understand their implication in ALS disease progression.

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B12 Nuclear mRNA export factor GANP in lower motor neuron degeneration

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Nuclear export defects have been recognized as an important cause of amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders. We recently identified a new recessive disease gene MCM3AP, encoding for the nuclear mRNA export factor GANP (germinal associated nuclear protein), in patients with early-onset peripheral neuropathy and mild intellectual disability. GANP is a scaffold for proteins of the TRanscription-EXport-2 (TREX-2) complex in the nuclear envelope, with a proposed role in exporting selective mature mRNAs from the nucleus. Intriguingly in flies, loss of the GANP ortholog xmas-2 was shown to suppress TDP-43-mediated toxicity and motor neuron degeneration. We studied skin fibroblasts of patients with GANP mutations, and found a marked reduction in GANP immunostaining in the nuclear envelope and by Western blotting. We further tested that GANP is a nuclear envelope protein also in cultured human neurons, and investigated the effects of GANP loss on axonal transcriptomes of neurons differentiated from patient-specific induced pluripotent stem cells. Our results show that recessive MCM3AP mutations cause a severe reduction of the GANP protein, suggesting disrupted mRNA export as the pathogenic mechanism. Understanding of the role of GANP in nuclear-cytoplasmic transport of mRNA in neuronal cells is important in regard to lower motor neuron degeneration in peripheral neuropathy and may also have implications for other motor neuron diseases associated with defective nuclear export such as ALS.

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B13 Bioenergetic profiling of SOD1 patient models of ALS

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Background It is well established that mutation in the SOD1 gene leads to oxidative stress and metabolic dysfunction in ALS. Metabolic defects have been observed in both patient and animal derived cell models of SOD1 such as fibroblasts and astrocytes. It is important to gain a better understanding of how SOD1 affects these pathways so they can be manipulated in order to try and increase energy production and slow down disease progression. Methods In order to identify novel sites of metabolic dysfunction. We have used a metabolic phenotypic array assay to profile patient derived fibroblast samples from 5 SOD1 ALS patients and 11 controls. This novel approach focuses on the production of nicotinamide adenine dinucleotides (NADH) from 91 different energy substrates using an OmniLogTM profiling system (Biolog). This approach uses proprietary redox dye technology which is reduced to a coloured product in the presence of NADH. Results Our on-going research has shown that in SOD1 fibroblasts there are disruptions in the energy substrates involved in the Tricarboxylic Acid Cycle, glycolysis and nucleoside metabolic pathways. Discussion We have reprogrammed these patient fibroblasts into induced neuronal progenitor cells (iNPCs) and are routinely differentiating these cells into astrocytes and performing metabolic profiling. Our aim is to elucidate if common dysfunctional pathways can be identified in a CNS specific human model of SOD1 ALS compared to fibroblasts and to identify novel areas of metabolic pathway dysfunction in astrocytes. Once we have identified these pathways we will supplement the astrocytes with the dysfunctional energy substrates in question. Or bypass the defect with energy substrates downstream of the dysfunction, to try to increase energy production.

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B14 Chaperone mediated autophagy respond to dynein mediated transport inhibition in motor neuron diseases

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The presence of protein aggregates in degenerating motoneurons represents a common hallmark of motoneuron diseases (MNDs), including amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA). Dysfunctions of the protein quality control (PQC) systems have been suggested to contribute to protein aggregation in MNDs. In NSC34 and motoneuron derived from iPS cells, inhibition of retrograde transport of misfolded protein mediated by dynein results in macroautophagy reduction and in co-chaperone BCL2-Associated Athanogene 1 (BAG1) mRNA increase. While dynein mediated transport is necessary for autophagy, BAG1 is responsible for misfolded protein degradation via proteasome. We have demonstrated that exogenous BAG1 overexpression reduced misfolded and aggregated species by proteasome degradation. BAG1 in association with HSPA8/HSC70 protein may also route misfolded proteins to chaperone-mediated autophagy (CMA) for degradation. CMA is a selective catabolic pathway responsible for the degradation of specific cellular proteins containing the KFERQ-like motif. After the recognition of this motif by HSPA8, substrates are translocated to the lysosomal receptor LAMP2A that allows the entry of substrates into the lumen of competent lysosomes. In NSC34 cells, filter trap assay showed a reproducible non-significant reduction of SOD1 and ARpolyQ insoluble species in response to dynein mediated transport inhibition when proteasome was inhibited. Interestingly, dynein mediated transport inhibition reduced ARpolyQ insoluble species, also when macroautophagy and proteasome are both inhibited. Dynein mediated transport inhibition did not modify the mRNA levels of all autophagy markers tested, but significantly increased Lamp2A mRNA and protein levels. Instead, HspA8 mRNA and protein remained unaltered. We demonstrated that dynein mediated transport inhibition reduced the Lamp1 protein levels. By overexpressing alpha-synuclein (SNCA), a well-established CMA substrate, we found that dynein mediated transport inhibition increased its clearance confirming that transport alteration may result in CMA response to clear misfolded protein. Collectively, these data show that inhibition of retrograde transport drastically impairs macroautophagy and this possibly prevents misfolded protein toxicity via CMA. Considering that CMA is a ubiquitous pathway, it is a conceivable that CMA can be used as a potential target to increase the clearance of misfolded proteins.

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B15 Neuron-specific non-canonical IFN-gamma pathway in ALS

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Background: Excitotoxicity is a key hallmark in ALS. Interferon-gamma (IFN-Ð) directly induces neurotoxicity in mice cortical neurons via formation of a calcium-permeable neuron specific receptor complex of IFNGR1 and GluR1. IFN-Ð control of calcium levels in motor neurons can provide a direct molecular link between neuroinflammation and neurodegeneration and pave way to therapeutic targets. Objectives: We aim to investigate (i) the distribution and (ii) expression level of IFNGR1 and GluR1 (iii) effects of IFN-Ð with and without kainate-induced excitotoxicity on (iv) neuronal survival and (v) cytosolic calcium. Methods: Motor neuron co-cultures from E13 mice spinal cords with (TG) or without (NT) hSOD1G93A were used for immunofluorescence. Also, cervical sections from adult mice were stained to determine basal levels of IFNGR1 and GluR1. qPCR was done for mRNA study. Cell survival was assessed after IFN-Ð treatment with or without kainate for 12 hours. Single-cell calcium measurements were done upon direct application of IFN-Ð. Results: Immunofluorescence and qPCR studies show similar levels of IFNGR1 and GluR1 in both NT and TG motor neurons in-vitro. However, ex vivo expression studies show overexpression of both the receptor units. Cell survival assay shows kainate as a strong agonist in motor neurons with IFN-Ð inducing weak neurotoxicity. This is supported by weak calcium signals in calcium measurements. Conclusion: Receptor expression studies show overexpression of IFNGR1 and GluR1 in adult TG mice motor neurons. Functional tests via cell-survival assay and calcium measurements support expression data from the motor neuron co-culture. Acknowledgment: This research is supported by a BMBF (the Bundesministerium für Bildung und Forschung) grant PYRAMID in the framework of the ERANET E-RARE program (http://www.e-rare.eu) and was undertaken in cooperation with the BMBF funded MND-NET.

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B16 TDP-43 protein and SUMOylation

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Post-translational modifications (PTM) of TDP-43 protein, including ubiquitinylation, phosphorylation and acetylation, have been described in ALS/FTD-affected brain tissues and seem to influence TDP-43 aggregation process. Among PTM, SUMOylation, consisting in SUMO proteins (1,2/3,4) conjugation to target lysine residues, controls a variety of biological activities, including protein stability, aggregation and nucleocytoplasmic transport, all important aspects in ALS/FTD diseases. Previous studies suggest that a short splicing isoform of TDP-43 can be SUMO-2/3-conjugated in cell insoluble fraction and that in response to heat shock TDP-43 is a target of SUMOylation, although the effects of this possible modification are still unknown. Here we investigated if TDP-43 is a substrate of SUMOylation and if this PTM can regulate TDP-43 function and/or aggregation in the cytoplasm. Our in silico analysis of TDP-43 amino acid sequence predicted Lys136 as the putative SUMO protein conjugation site, and the 106-110 amino acid region as a potential SUMO-interacting motif by a non-covalent binding. By immunoprecipitation assays we proved that a fraction of TDP-43 protein is physiologically SUMOylated in HEK293 and in human neuroblastoma cells in its RRM1 domain. We also showed that TDP-43 SUMOylation levels and sub-cellular distribution between nucleus and cytoplasm compartments can be modulated upon over-expression of UBC9 and SENP1 SUMOylation enzymes. The SUMO-resistant TDP-43 K136R protein showed a defective splicing activity of known target transcripts and a diminished RNA binding capability by UV-cross-linking assays. Moreover, upon exposure to Arsenite insult the SUMO-resistant TDP-43 K136R protein was not able to be recruited into stress granules in contrast to TDP-43 proteins with mutations identified in ALS/FTD patients in the C-term region (Q331K, M337V, A382T). Our preliminary results show that SUMOylation is a physiological TDP-43 PTM, potentially influencing its RNA-binding and splicing activity, and therefore deserves further investigation as a potential mechanism regulating not only TDP-43 function, but also its aggregation, in ALS/FTD diseases.

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B17 Regulation of exosome secretion to diminish toxicity of the muscle secretome in ALS myotubes

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder characterised by degeneration of both upper and lower motor neurons, culminating in muscular atrophy, paralysis and ultimately, death. Research demonstrates skeletal muscle to show functional secretory behaviour and work in our lab has identified abnormalities in the secretory activity of ALS muscle. Exosomes are endosome derived vesicles that carry various proteins, lipids and genetic materials capable of altering the function of neighbouring cells. ALS muscle can be seen to secrete exosomes at a measure that is 2-fold more than that secreted by healthy and other NMD controls. Furthermore, addition of ALS exosomes to the culture medium of healthy myotubes and iPSC motor neurons leads to increased cell death compared to cells treated with exosomes secreted by healthy control myotubes. Aims: We hypothesize the presence of toxic constituents within exosomes secreted by the skeletal muscle of ALS patients. We therefore aim to assess the effects of altered exosome biogenesis and secretion on cell survival, and subsequently to target the expression of toxic cargoes. Methods: Immortalized myoblasts were used to optimize concentrations of secretion-regulating reagents. Exosome secretion was blocked using dimethyl amiloride (DMA), or stimulated with Monensin (Mon), by addition of either reagent to myoblast culture medium for an incubation period of 72 hours during differentiation. Cell viability was assessed using nuclear staining (DAPI) and culture medium was harvested for later exosome isolation and protein quantification. Results: Relative to vehicle only (MeOH) treated myotubes, treatment with DMA at high concentrations was toxic, but lower concentrations were observed to reduce exosome secretion without affecting cell death. Cytosolic levels of the exosomal membrane marker, CD63, did not differ between conditions. Application of a non-toxic dose of Mon resulted in increased secretion of exosomes. Furthermore, Mon-treated myotubes were smaller, a feature observed in cultured ALS myotubes. Conclusions: Treatment with DMA reduces exosome secretion in immortalized myotubes, whereas stimulating exosome secretion using Mon recapitulated aspects of the ALS signature observed in ALS myotubes. Further experiments will elucidate whether chemical regulation of exosome secretion alters exosome toxicity and may provide insight into the role of muscle derived vesicles in ALS.

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B18 Perinuclear accumulation of SOD1 in sporadic ALS myotubes, and its impact on cell-cell communication

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Objective: The antioxidant Cu, Zn superoxide dismutase 1 (SOD1) has been shown to have prion-like properties, and to aggregate in affected neurons of sporadic Amyotrophic lateral sclerosis (ALS) patients. In this study, we investigated inter-cellular diffusion of SOD1 aggregates in sporadic ALS muscle cells. Background: ALS is characterized by progressive degeneration of both lower and upper motor neurones and is associated with muscle atrophy and mitochondrial dysfunction. While the mechanisms of pathogenesis and spread of disease in the body remain unknown, several lines of evidence indicate: (1) the involvement of muscle, and (2) a prion-like effect, with the transmission of misfolded proteins being responsible for the propagation of clinical manifestations throughout the body. Methods: Primary muscle stem cells were extracted from the deltoid muscle biopsies of sporadic ALS patients (n=4) and aged-matched healthy subjects (n=4) and were immortalized by overexpressing hTERT and Cdk4. Exosomes from ALS and healthy muscle cells were extracted using exosome kit (LifeTechnologies®). Immunostainings and western blots were performed on muscle stem cells and muscle exosomes to investigate SOD1 aggregations and secretion. Results: We consistently observed an aggregation of SOD1 in sporadic ALS myotubes. SOD1 aggregates surrounded the myonuclei, and co-localized with the mitochondria. Together, these data suggest that SOD1 is affected in ALS, independent of SOD1 mutation, and may lead to mitochondrial dysfunction. We occasionally found a co-localization of SOD1 aggregates with the exosomal marker CD63. Consistently, we did not observe SOD1 in secreted human exosomes. When ALS exosomes were added to the culture medium of healthy muscle cells expressing WT-SOD1-FLAG, no SOD1-FLAG aggregation was induced. Conclusion and perspectives: These findings implicate SOD1 in cellular pathology of ALS muscles, and suggest that SOD1 aggregation in muscle is independent or downstream of exosome propagation. It will be interesting to now investigate the potential role of microparticles in the propagation of SOD1 aggregates.

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B19 Paraspeckle-like properties of G4C2 RNA foci

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The expansion mutation of the GGGGCC repeat in the gene C9ORF72 is the most common genetic cause of FTLD and ALS. It is transcribed both from the sense and the antisense strands leading to the formation of nuclear RNA foci, which may sequester specific RNA binding proteins and affect various steps of post-transcriptional gene regulation. Core paraspeckle proteins SFPQ, NONO and PSPC1 bind to (G4C2)n repeat RNA in vitro, and colocalize with nuclear RNA foci in transfected cells and brain tissue of C9ORF72 mutant carriers at post-mortem. G4C2 RNA foci lead to an increased number of SFPQ-stained subnuclear bodies, which form independently of the known paraspeckle platform long non-coding RNA NEAT1. Furthermore, (G4C2)72 RNA foci also colocalized with paraspeckle-associated associated Alu repeat-containing RNAs, indicating that (G4C2)n RNA foci might replace NEAT1 as scaffold of paraspeckle-like structures. Our results suggest that (G4C2)n RNA foci form paraspeckle-like structures, which function in similar fashion as paraspeckles and modulate nuclear compartmentalization of paraspeckle-bound RNAs.

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B20 ALS associated mutations impair AchR clustering in skeletal muscle

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by degeneration of upper and lower motor neurons (MNs). This leads to muscle weakness and eventually respiratory failure. In addition to MNs, the pathophysiology in ALS may also involve the skeletal muscle. In a motor unit, the MN connects to the muscle via the neuromuscular junction (NMJ), during development MNs are potent inducers of acetylcholine receptors (AchR) clustering to form mature NMJs. We tested the hypothesis that ALS related mutations affect AchR clustering. To that end, we examined peripheral synaptic contacts of motor units in neuron-muscle co-cultures derived from human induced pluripotent stem cell (hiPSC) lines from controls, FUS and TBK1 ALS patients. MNs expressing ALS mutations were less potent in inducing mature AchR clusters on myotubes from unaffected controls. ALS derived myotubes, cultured either with healthy MNs, or without MNs, AChR clustering was even more impaired as evidenced by a higher proportion of immature AChR clusters. When ALS mutant MNs and mutant myotubes were co-cultured, as in a patient context, only immature or aberrant AchR clusters were seen. We provide evidence that ALS associated mutations impair maturation of NMJ in single cultured skeletal muscles, as well as when co-cultured with MNs.

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B21 Mitochondrial location of nuclear proteins: A common mechanism for ALS-related cellular stress?

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The presence of cytosolic aggregates of selected nuclear proteins is a hallmark for ALS pathological diagnosis. Previous evidence links the formation of these protein inclusions to cellular stress such as oxidative, endoplasmic-reticulum, proteasomal or mitochondrial stress. Interestingly some of these nuclear proteins exhibit a mitochondrial location. In order to establish whether the potential mitochondrial translocation of these nuclear proteins could play a role in its ulterior cytosolic aggregation, we have performed a systematic high-content screening based on epithelial cell culture, confocal microscopy and software-assisted image analyses. We treated cells with hidrogen peroxide, thapsigargin, epoxomycin and rotenone at different concentrations and time to recreate the abovementioned stresses. Then, we have quantified by image analysis changes in the amount of phospho-TARDBP, phospho-ERK, REST and phospho-Jun in nuclear, cytosol and mitochondrial compartments of these cells. Our results demonstrate significant changes in nuclear/cytosolic ratio of these proteins when comparing stressed cells with non-stressed ones. This is also true for the degree of their mitochondrial translocation. However, the sense of change depends on the protein. For instance, short-term oxidative burst induces an increase in the cytosolic amount of phospho-TARDBP (76.73%) and REST (30.01%), while it decreases phospho-Jun (5.77%) and phospho-ERK (82.62%), suggesting a potential role of oxidative stress in proteostasis alterations in ALS. Also, the degree of colocalization of these proteins with mitochondrial epitopes (ATP5A) also increases (ranging from 25.58% for phospho-Jun, 58.64% for REST, 224.8% for phospho-ERK and 245.87% for phosphor-TARDBP) after oxidative stress . We have explored these features for each of the applied stresses, and globally proteasomic and oxidative stresses impinge the more drastic changes in comparison with other stress inducers. Our results suggest that an homeostatic trait (i.e. increased amount of stress-response proteins and mitochondrial translocation) could sensitize cells for development of proteostasis alterations, increasing the chances for aggregation.

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B22 Modeling and mechanistic insights in C9orf72-mediated neurodegeneration

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Intronic GGGGCC repeat expansions in C9orf72 are considered the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Two major pathologies stemming from the RNA expansions have been identified in postmortem tissue: intracellular RNA foci and repeat-associated non-ATG dependent (RAN) dipeptides, though it is unclear how these and other hallmarks of disease contribute to the pathophysiology of neuronal injury. Here, adeno-associated virus (AAV) was used to model C9orf72 pathology in neuronal cells and rodent. We report that AAV encoding 10 pure, 102 interrupted GGGGCC repeats or 69 V5-tagged DPRs delivered into the cerebrospinal fluid via cisterna magna of wild type mice led to transgene expression in multiple areas of the CNS and cause neurodegenerative phenotype[1.2]. Virus-mediated expression of C9orf72-related RNA and dipeptide repeats in the mouse central nervous system increases double strand breaks and ATM defects and is associated with neurodegeneration[1]. We also report elevated levels of DNA-RNA hybrids (R-loops) and double strand breaks in rat primary neurons and C9orf72 ALS patient spinal cord tissues. These findings suggest that C9orf72-mediated neurodegeneration is driven, at least partly, by genomic instability. References 1. Walker et al., Nat Neurosci. 2017; 20(9):1225-1235. doi: 10.1038/nn.4604. 2. Herranz-Martin et al., Dis Model Mech. 2017; 10(7):859-868. doi: 10.1242/dmm.029892

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B23 Neuregulin 1 reduces motoneuron cell death and promotes neurite growth in an in vitro model of motoneuron degeneration

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder with no effective treatment currently available. Although the mechanisms of motoneuron (MN) death are still unclear, glutamate excitotoxicity and neuroinflammatory reaction are two main features in the neurodegenerative process of ALS. Neuregulin 1 (NRG1) is a trophic factor highly expressed in MNs and neuromuscular junctions. Several recent evidences suggest that NRG1 and their ErbB receptors are involved in ALS. However, further knowledge is still needed to clarify the role of the NRG1-ErbB pathway on MN survival. In this study we used an in vitro model of spinal cord organotypic cultures (SCOCs) subject to chronic excitotoxicity caused by DL-threo-Ð-hydroxyaspartic acid (THA) to characterize the effect of NRG1 on MN survival. Our results show that addition of recombinant human NRG1 (rhNRG1) to the medium significantly increased MN survival through the activation of ErbB receptors which was ablated with lapatinib (LP), an ErbB inhibitor, and reduced microglial reactivity overcoming the excitotoxicity effects. rhNRG1 activated the pro-survival PI3K/AKT pathway and restored the autophagic flux in the spinal cord culture. Moreover, addition of rhNRG1 to the medium promoted motor and sensory neurite outgrowth. These findings indicate that increasing NRG1 at the spinal cord is an interesting approach for promoting MN protection and regeneration.

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B24 Neuroimaging needs time to shine: Structural brain involvement in a multimodal longitudinal study in ALS

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There is a growing interest in identifying patterns of neurodegeneration over time to better understand the progressive nature of ALS. Previous cross-sectional neuroimaging studies have reported brain abnormalities in ALS patients extending the motor regions. Longitudinal neuroimaging studies are required to document cerebral changes that occur during disease progression. We investigated cerebral changes over time in patients with ALS using a multimodal approach. In total, 292 ALS patients, including 24 with a C9orf72 repeat expansion, and 156 controls participated. From T1- and diffusion-weighted MRI data, cerebral measures in terms of cortical thickness, subcortical volumes and white matter connectivity were assessed. The median disease duration at baseline was 13.03 months. Follow-up data was available from 150 ALS patients and 72 controls, with median follow-up time between the first and second visit of 5.23 months. Changes in structural brain measurements over time were assessed using linear mixed-effects models. Compared to controls, ALS patients showed progressive cortical atrophy of primary motor and frontotemporal regions, smaller basal ganglia volumes, ventricle enlargement, and a reduced fractional anisotropy (FA) of a largest connected component (LCC), linked to the motor cortex (all $p<0.05$). ALS patients with bulbar onset showed involvement of frontotemporal regions at baseline, whereas these regions showed atrophy later in time for the patients with spinal onset. The LCC in patients with spinal onset already included these frontotemporal regions at baseline. Regarding disease duration, patients with longer disease duration had a considerable lower FA and cortical thickness at baseline, while additional changes in time were mostly found in the patients with the shortest disease duration. Longitudinal imaging in patients with a C9orf72 repeat expansion was characterised by extensive loss of white matter integrity over time, while grey matter was already extensively affected at baseline and showed no additional changes over time. Neuroimaging captures longitudinal cerebral changes related to ALS and provides a tool for investigating neurodegeneration in ALS in vivo. The multimodal approach reveals different patterns of grey or white matter involvement that is related to phenotypic heterogeneity. These results might be valuable in tailoring study design by selecting appropriate neuroimaging techniques as a biomarker to study disease progression.

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B25 Utilizing network medicine approaches to explore the role of muscle in ALS

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Amyotrophic Lateral Sclerosis (ALS), is a progressive and fatal neuromuscular disorder and the most common form of motor neuron disease (MND). The pathology is complex and multifactorial with many dysregulated physiological processes being identified to contribute. Recent evidence suggests that muscle could play an important role in disease pathology, and muscle is implicated in the neuronal dying back hypothesis. With this in mind, we will incorporate network medicine approaches to explore the role of muscle in ALS. We hypothesise that a mechanistic pathway in muscle cells is shared by ALS-associated genes. Furthermore, that these functional effects of ALS pathology, in muscle cells, can be understood in terms of changes to the behaviour of molecular interaction networks. To test this hypothesis, we will construct molecular interaction networks relevant to muscle tissue, and attempt to discover one or more functional modules linked to ALS-dysregulated or associated genes or genes carrying common genetic variants observed in sporadic patients. Muscle specific networks have been constructed using the MyoMiner muscle gene co-expression database (http://sys-myo.com/myominer/), while tissue-type-generic networks have been obtained from the STRING protein database for protein-protein interactions, and the GTEx Portal for gene co-expression. We are investigating the ability of each network to form disease modules or links between differentially expressed genes (DEGs) from ALS myotubes, known MND associated genes and muscle specific disease gene lists. To test network suitability, the Disease Module Detection Algorithm (DIAMOnD) will be used to help identify the network or combination of networks having the best linkage between these disease genes for each condition. Disease modules associated to ALS may suggest novel genes and pathways as biomarker and therapeutic candidates in ALS pathology.

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B26 Dying-forward or dying-back – tract-type specific fractional anisotropy as a potential biomarker for ALS

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Background: Facing the rise of various biomarkers for amyotrophic lateral sclerosis (ALS), the pathological processes of dying-forward and dying-back hypothesis are still controversial. The aim of this study was to test the dying-forward and dying-back hypothesis with fractional anisotropy (FA) of different white matter (WM) types: brainstem tracts, projection fibers, association fibers, and commissural fibers. Methods: The sample included 24 limb onset classical ALS patients (10 females, M=48 yrs, SD=11 yrs, Mean ALSFRS-R score: 37, SD=7) compared to 27 age-related healthy controls (M=43 yrs, SD=10yrs). ALS patients were treated by standard Riluzole and additional longterm G-CSF (Filgrastim) on a named patient basis with written informed consent. Diffusion weighted imaging enabled the extraction of FA for different types of white matter tracts defined by a WM atlas (Mori et al. 2008). Patientsâ€™ raw fractional anisotropy values of 48 regions of interest (ROI) were compared to age-related healthy control groups resulting in z-transformed deviations. Alterations in FA of brainstem tracts, projection, association, and commissural fibers were observed in cross-sectional group analysis at initial MRI scan (n=24) and monitored longitudinally over the time course of the disease (n=21). Results: Cross-sectional group analysis revealed the most pronounced FA alterations in the brainstem tracts. Moreover, analysis of the individual levels of FA deviations in every patient also showed a subset of patients with more pronounced FA alterations in the projection, association or commissural fibers than in brainstem tracts. Longitudinal monitoring of FA deviations in 21 patients demonstrated the spread of degeneration across different types of white matter tracts in forward as well as backward directions. Discussion: Results of both cross-sectional and individual longitudinal monitoring of FA deviations of ALS patients argue for both spread of disease from brainstem to projection, association and commissural fibers and vice versa.

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B27 Secretion of toxic exosomes by muscle cells of ALS patients: Interaction with FUS

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Background: As several studies show that motor neuron degeneration starts at the neuromuscular junction and as we have previously shown that skeletal muscle can have a functional secretory activity, we hypothesized that ALS muscle cells can release vesicles such as exosomes and participate to motor neuron death. Objectives: The purpose of the present study is to determine whether intercellular communication between muscle and nerves is altered and could thus have a key role in ALS pathogenesis. Design & methods: Muscle stem cells were extracted from the deltoid muscle biopsies of sporadic ALS patients and aged-matched healthy subjects (n=18/21 per group). Human Pluripotent Stem Cells were converted into pure populations of spinal motor neurons as previously described. Exosomes from ALS and healthy muscle cells were extracted using exosome kit (LifeTechnologies®). Results: In muscle of sporadic ALS patients, we observed (1) multi-vesicular bodies that were filled with xosomes (1.40 ± 0.14 exosomes/mm2 in ALS, 0.9 ± 0.07 exosomes /mm2 in control), and (2) cultured ALS muscle cells had massively increased exosome content – as shown by RT-qPCR and immunostaining - and released 2-fold more exosomes. These exosomes are toxic to both muscle cells and motor neurons as once added to the culture medium of healthy muscle cells or of healthy motor neurons, they induced: (1) muscle fiber atrophies, (2) cellular stress by stimulating blebbings, and (3) cell death of muscle cells and motor neurons. When ALS exosomes were added to the culture medium of human muscle cell line that over-expresses a tagged form of wild type FUS (FUS-FLAG), it induced markedly greater cell stress and death than when added to SOD1-FLAG or TDP43-FLAG cell lines. These data suggest an interaction of ALS exosome content with FUS. Interestingly, FUS is aggregated in the nuclei of ALS muscle cells. Taken together, these data suggest that FUS function is impacted in ALS muscle cells, and that the toxic effect of ALS exosomes is exacerbated in the presence of FUS over-expression. Conclusion: The present study suggests that muscle cells from sporadic patients secrete toxic agents through their exosomes – agents that interact with the FUS pathway. These exosomes may influence the intercellular communication between the muscle and its environment, including motor neurons, and may have a key role in the pathology of ALS.

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B28 Peak cough flow is a good biomarker that correlates with disease progression and survival in ALS

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Background: Cough efficacy assessment is of clinical relevance in different neuromuscular diseases, including ALS. The ALSFRS-R provides an estimation of the functional impairment, which can be evaluated to assess any response to treatment or progression of disease. Changes of total ALSFRS-R score over the time (ΔFS) is considered a better indicator of the rate of progression than the total ALSFRS-R score alone. Objectives To evaluate the relationship among peak cough flow (PCF) and measures of functional impairment, disease progression and survival. Methods PCF, ALSFRS-R total score and its bulbar (ALSFRS-Rb) and respiratory (RofALSFRS-R) subscores were assessed at the first evaluation (T0) and after 6 months (T1). ΔFS was calculated as: (48 - ALSFRS-R at time of the evaluation)/disease duration from onset to evaluation (month). Monthly decline of PCF and ALSFRS-R was calculated as the differences between the T1 – T0 values / time from T0 to T1. Survival was defined as time from onset to tracheostomy, death or censoring data. We studied baseline and longitudinal correlations. Results 73 patients (mean age: 62.44 ± 9.93 yrs; M/F ratio: 52/21; type of onset: limb: 86%, bulbar: 14%) were studied. Baseline analysis showed significant correlations between PCF and ALSFRS-R total score (p<.0001; r=0.49010), ALSFRS-Rb (p=0.0004; r=0.40835) and ΔFS (p=0.0008; r=0.35018). We confirmed the same results also in longitudinal data. In fact, dividing our sample in two groups based on the median value of PCF monthly decline (-5.96 l/min for month), we found that the group with the worst monthly decline showed a significant median faster decline also in ALSFRS-R total score (-0.81 [-1.42 – -0.34] vs -0.34 [-0.89 – 0.00]; p=0.0039), ALSFRS-Rb (p-0.17 [-0.35 – 0.00] vs 0 [-0.17 – 0.00]; p=0.0170) and disease progression (0.68 [0.44 – 0.98] vs 0.45 [0.27 – 0.70]; p=0.0150). A significant relation with survival, using PCF at baseline as continuous variable, was also observed (p=0.0221). Conclusions PCF represents a non-invasive maneuver of cough efficacy. An impaired cough predisposes to chest infections with an increased mortality risk. Our study showed that the PCF is a good biomarker for prognosis in ALS patient with a significant correlation with disease progression and survival. PCF decline is a sensitive measure of global functional deterioration over time in ALS and may be relevant in the routine and experimental evaluation of these patients.

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B29 Brain morphology is associated with C9orf72 mutation and regional gene expression

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Background: A GGGGCC repeat expansion (RE) in the C9orf72 gene is identified as a highly penetrant mutation in familial ALS and FTD. The C9orf72 RE is also associated with disease progression and poor survival. The mechanism underlying C9orf72 RE associated ALS and cerebral changes in (a)symptomatic carriers, however, remains unclear. Objective: To study the effect of C9orf72 RE and gene expression on brain MRI to comprehend its implication in the development of ALS. Methods: We included 189 ALS patients, 97 family members without ALS/FTD (FCO) and 114 controls. Subjects were subdivided in these groups by the presence of a C9orf72 RE (C9+ or C9-). Participants underwent 3T MRI scans longitudinally. Brain measurements were assessed using T1- and diffusion-weighted MRI data. A profile of regional cortical thickness, subcortical volume and white matter fractional anisotropy (FA) was derived for each group using linear mixed models. We corrected for multiple comparisons using family-wise error rate correction. Cortical C9orf72 gene expression was obtained from the Allen Human Brain Atlas, including RNA microarray data collected from post-mortem brains of six donors without history of psychiatric or neuropathological disorders. Expression levels were normalized across samples and donors. The relationship between C9orf72 expression and relative cortical thickness ('thinning') was evaluated using Pearson correlation analysis. Results: We found a significantly thinner cortex in 19 regions and smaller subcortical volumes for 3 structures when comparing C9+ and C9- FCOs. Between C9+ and C9- ALS, we found a thinner cortex in 40 regions and smaller subcortical volumes for 7 structures. There were no differences in FA. No significant changes were found during follow-up scans. There was a negative correlation between C9orf72 gene expression and cortical thinning between C9+ and C9-, in both FCO ($r=-0.36$, $p=0.035$) and ALS groups ($r=-0.62$, $p<0.001$). A positive trend was found in the ALS C9- group compared to controls ($r=0.28$, $p=0.108$). Discussion: C9orf72 RE carriers show thinner cortex and smaller subcortical volumes compared with non-carrier family members, indicating effects of the C9orf72 RE on the brain. Our observation of gene expression to be correlated with cortical thinning suggests that not only the RE mutation itself, but also the expression of C9orf72 is associated with cortical thickness and thus might be involved in the pathogenic mechanism underlying ALS.

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B30 Prediagnostic elevated levels of phosphorylated neurofilament heavy chain in blood of patients with amyotrophic lateral sclerosis

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Background: The diagnostic delay in patients with amyotrophic lateral sclerosis (ALS) is about one year, which represents a significant proportion of the overall disease duration. Biomarkers that identify neurodegeneration at an earlier disease stage might decrease the diagnostic delay. We showed before that levels of phosphorylated neurofilament heavy chain (pNfH) are elevated in blood of patients with ALS at time of diagnosis. We now assessed whether those findings could be extended to an earlier stage of the disease. Methods: Seventy-two patients with an established diagnosis of ALS were included in this retrospective study. For each patient, leftover of serum, drawn for routine purposes well before the time of diagnosis, was retrieved from the biobank of our hospital. An enzyme-linked immunosorbent assay was used to determine serum pNfH concentrations (Euroimmun AG, Lübeck, Germany). Results: The median disease duration at blood sampling and diagnosis was 6.5 months (range: -18.3 to 36.1) and 10.6 months (range: 2.0 to 40.7), respectively. In sixty-three percent of the prediagnostic serum samples pNfH was higher than our previously established diagnostic cutoff of 81.9 pg/mL. On average, serum pNfH was already significantly increased up to 11.2 months before the diagnosis of ALS. Furthermore, in prediagnostic serum samples, pNfH was a predictor of survival with age and gender as covariates (Hazard ratio: 2.87, 95% Confidence interval: 1.28 â€“ 6.44). Conclusion: Our retrospective findings demonstrate that pNfH in serum is elevated well before the diagnosis of ALS. Therefore, our findings encourage to prospectively explore if pNfH in serum could decrease the diagnostic delay in patients with ALS.

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B31 Reading the patient's palm – The contrary pattern of hand muscle denervation in ALS and SMA

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Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are motoneuron diseases which share a pathological hallmark, the dying back phenomenon of the lower motoneuron. This results in denervation of neuromuscular junctions with consecutive muscle weakness and atrophy. While we know that ALS has different clinical phenotypes at the onset of disease, the phenotype of SMA is widely described as primarily proximal. However, sooner or later, weakness of distal muscles can be observed in ALS as well as in SMA. For ALS he term 'split hand' was used for the prominent weakness/atrophy of the thenar muscle group in comparison to a relatively spared hypothenar group. In contrast, we found that patients suffering from SMA had a relatively spared thenar group with a persistent ability to abduct the thumb even in severely affected adult patients. We analyzed and compared electrophysiological data (CMAP, MUNIX, MUSIX) of the hand muscles (APB, ADM, FDI) of ALS, SMA and healthy controls to validate the clinically observed variability of hand muscle weakness. Our data suggest a disease dependent selective vulnerability of different lower motoneuron pools in the spinal cord, which leads to contrary pattern of hand muscle denervation in ALS and SMA. We therefore hypothesize that the type of motoneuron disease could be 'read in the patient's palm'.

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B32 Chromosome conformation signatures as a clinical tool for diagnosis, prognosis and disease understanding in ALS

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Only 10% of ALS patients (fALS) have a known genetic mutation that can be named as the contributing factor to the development of disease. For the remaining 90% the causes are much less clear with many physiological pathways and environmental factors implicated. Chromosome conformation signatures (CCSs), which define the initial regulatory process in integrating environmental cues into the epigenetic and transcriptional machinery, can be utilised to monitor the effects of multiple factors on disease phenotype. Here we report the identification and development of CCS based on a high throughput microarray and PCR based discovery pipeline (EpiSwitchTM) for the diagnosis and prognosis of ALS patients. For the study a total of 100 patients, presenting to the NDCN, enrolled and asked to return at 3 and 6 months. Healthy controls (n=100) were also collected. During each visit, participants underwent the ALS-FRS-R and FVC tests, and provided a blood sample. The samples were analysed to identify either, an ALS- disease-related diagnostic signature (at presentation), or the prognostic disease-related signature (ALS patients at 3 and 6 months). Results of the clinical assessments were compared to the EpiSwitchTM analysis at 0, 3 and 6 months. A cut off of a 0.5-point decline per month of the ALS-FRS-R score was used to cluster the ALS patients into progression-subtypes.

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B33 Could biochemical parameters and/or comorbidities support the prognosis of ALS?

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Introduction: Amyotrophic Lateral Sclerosis is a neurodegenerative disease which involves the upper and lower motor neurons. Several molecular markers have been identified as potential prognosis factors and as a support for diagnosis. Objectives: the aim of this work is to study the molecular features of a cohort of ALS patients, in order to determine which of them predict different patterns of survival. Methods: The data was compiled retrospectively from 110 ALS patients registered in the neuromuscular consultation of the Hospital Clínico Universitario Lozano Blesa of Zaragoza from 1995 to the present day. Results: Based on the age or the gender, the findings obtained were different according to the biochemical parameters. Increasing levels of creatinine ($p<0.005$) and creatinine-phosphokinase ($p<0.05$) in men were correlated with a higher survival. In relation to the age, in patients above 63 years high levels of albumin were correlated with a longer survival rate ($p<0.01$). Regarding to comorbidities, no differences in age and gender were observed. All the patients diagnosed of mental disease or head trauma were indicators of bad prognosis. Discussion: The results suggest that biochemical parameters usually monitored at clinical level, and the clinical history can be of help to estimate the progression of the disease. However, prognosis must be analyzed individually according to each patients age and gender. Further studies are necessary to better understand why these parameters and comorbidities can influence disease progression. Conclusions: In our cohort of patients, high levels of creatinine and creatinine-phosphokinase were found reliable prognostic markers of disease progression in men. In men and women, patients above 63 years high levels of albumin were found reliable prognostic markers of disease progression. Moreover, a medical history mental disease and head trauma are indicators of bad prognosis. These findings should be taken into account in clinical care. Key words: ALS, Motorneuron disease, survival, prognostic factor.

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B34 Functional interhemispheric connectivity of motor cortices in ALS using EEG source analysis

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting motor neurons [1] characterised by abnormal functional connectivity in motor networks. Our recent quantitative EEG studies have confirmed fMRI findings of increased connectivity in the left motor cortex (LMC) [2] and our diffusion tensor imaging studies have shown structural degeneration of white matter tracts in the motor-associated part of the corpus callosum (CC) [3]. Further investigation is required to elucidate disease pathophysiology by identifying the nature and patterns of connectivity change. This study further characterises the altered interhemispheric connectivity between left and right motor areas in ALS using both amplitude- and phase-based measures of functional association in the source space. Methods: Resting-state EEG (128 channels) was recorded for 6 minutes from 150 ALS patients and 40 healthy controls. Linearly Constrained Minimum-Variance beamformer was used to estimate source signals in 6 predefined points in the MC (3 left; 3 right). Connectivity was assessed in 8 frequency bands using amplitude envelope correlation (AEC) and imaginary coherence (IC). Results: The analysis of a subset of 33 patients and 13 controls to date, identified statistically significant decrease (Mann-Whitney U-test, $p = 0.003$; adaptive false discovery rate, $q = 0.05$) in interhemispheric AEC in ALS patients in high beta-band. Similarly, we found marginally significant changes ($p = 0.034$, $q = 0.05$) in IC in low alpha-band. Discussion: Slow co-modulation of the activity of sources can be measured using AEC. These fluctuations are likely common neuromodulatory systems in the brainstem; hence, the decrease of AEC in high beta-band in ALS supports previous findings of neuronal degeneration and dysfunction in the brainstem [4]. In addition to this, the IC results suggest impaired low alpha-band phase synchrony between motor cortices in ALS patients that could be attributed to degeneration of the CC. 1. O. Hardiman et al., Nat. Rev. Neurol., 7: 639-649, 2011. 2. B. Nasseroleslami et al., Cereb. Cortex, 1-15, 2017. 3. Bede, P. & Hardiman, O., Amyotroph. Lateral Scler. Front. Degener., 1-10, 2017. 4. B. R. Foerster et al., Nat. Rev. Neurol., 9:513-24, 2013.

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B35 Optometric analysis in amyotrophic lateral sclerosis patients

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Background: During later stages of ALS, having clear vision and functional ocular motility is one of a key aspect for the patient who usually communicate with eye-tracking system. The visual system is generally preserved in ALS patients, but some signs of eye dysfunction have been detected and reported in recent papers. Most scientific studies focus on ocular motility: pursuit, nystagmus and saccadic impairments are observed. Also retinal morphology is a matter of scientific interest in this kind of disease. However, there are few studies that correlate standard optometric analysis outcome and ALS. The aim of this study is to perform optometric tests on ALS patients and evaluate the relation between ocular data and disease clinical features. PATIENTS AND METHODS: 114 ALS patients (mean age: 62.98 ± 12.32; M/F 66/48) are involved in the study. The optometric protocol included an ocular history and symptoms questionnaire, ocular motility test (NSUCO test) and near point of convergence (NPC), the error refraction measurement, visual acuity, heterophoria and heterotropia assessment and fusional vergences. The relation between the optometric tests and the clinical features (disease progression rate, ALSFRS-R, type of onset and time from onset to evaluation) was investigated using the Student's t test and the non-parametric Wilcoxon rank-sum test for continuous outcomes, and the chi square test for the categorical and dichotomous ones. RESULTS: Ocular discomfort was significantly more frequent when the disease duration exceeds 24 months: in particular we reported tear alteration (p=0.02), photophobia (p=0.02), ocular tiredness (p=0.04) and burning eye sensation (p=0.02). Tear alteration and ocular tiredness were also related to ALSFRS-r, indeed these two symptoms were more often reported by patients with ALSFRS-r score lower than 29, which is the median value of our population (p=0.02, p=0.05, respectively). Saccades accuracy becomes worse for disease progression rate higher than 0.45, which is the median value of our sample (p=0.03). DISCUSSION: Our results show that some aspects of the visual system are involved in ALS disease. Among the different reported symptoms, tear alteration seems to be the most critical aspect. It will be interesting to analyse the tear film through more precise qualitative and quantitative techniques. Besides, saccades seem to reveal some information about the disease evolution.

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B36 Assessing cortico-muscular communication in motor neuron disease

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Background: There is emerging evidence of non-motor and motor impairment beyond the motor neurons in MND/ALS. Assessment of impaired network communication can provide a better understanding of the underlying disease mechanism and the possible distortion in the central-peripheral communication in the motor system. Recording of electroencephalogram (EEG)[1] and electromyogram (EMG)[2] for time-series analysis[3] can quantify the level of communication between cortical brain regions while quantifying the oscillatory motor drives of muscles. The study of cortico-muscular coherence (CMC) between EEG-EMG during motor tasks aims to inform on the specific alterations within and beyond the primary motor (M1) cortex in ALS. Method: High-density 128-channels EEG and 8 bipolar surface EMG recordings from hand muscles were obtained from 10 patients with dominant upper (primary lateral sclerosis), lower (Poliomyelitis) and mixed upper/lower (amyotrophic lateral sclerosis) motor neuron degeneration as well as on 3 healthy controls, during isometric precision grip tasks. Results: Data analysis at the individual level shows that frequency, location and intensity-specific features of CMC can distinguish the individual patients from healthy controls. Preliminary analysis at the group level suggests significant band-specific abnormal changes in the frontal and parietal cortices of all the 3 groups. Discussion: Previous studies in ALS indicate a dominant degeneration in corticospinal tract[1,4], which could affect the CMC over M1 areas. However, the detection of abnormal patterns of CMC in other regions than M1 may reflect a compensation for loss of normal M1 corticospinal projections. Further analysis is required to fully characterize the pathological CMC in ALS. The distinct signatures exhibited by the 3 groups of patients suggest that this methodology may provide a means of unmasking altered neural communication. This is of potential use as a neurophysiology-based stratification parameter and biomarker in motor degenerations. Moreover, the changes in the post-polio cohort suggest the presence of previously unrecognized functional changes in supra-spinal networks in pure lower motor neuron syndromes, which could be of utility for studies on spinal muscular atrophy. 1. Iyer PM et al PloS one. 2015;10(6);e0128682. 2. Fisher KM et al. Brain. 2012;135(Pt 9):2849-64. 3. Halliday DM et al. Prog Biophys Mol Biol.1995;64(2-3):237-278 4. Nasseroleslami B et al. Cereb Cortex.

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B37 Combined metabolomics and lipidomics analyzes of fibroblasts from ALS patients

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Although genetic and environmental factors have been associated with Amyotrophic Lateral Sclerosis (ALS), pathophysiology remains unknown in the majority of cases. Previous findings from our group revealed metabolomics and lipidomics signatures of ALS, based on (cerebrospinal fluid) CSF samples and cellular models exploration. In order to better characterize the metabolic alterations observed in ALS, we explored metabolome and lipidome of fibroblasts from ALS patients and subcellular fractions containing mitochondria and endoplasmic reticulum (mito-ER). We also assessed mitochondrial oxidative respiration and whole mitochondrial genome (mtDNA) was sequencing. We compared fibroblasts' signatures of 10 ALS patients to 10 matched controls, using both univariate and multivariate statistical analyzes. We detected and identified approximatively 130 metabolites and 300 complex lipids in fibroblasts and purified mito-ER fractions. Using multivariate analysis, we observed specific metabolomic and lipidomic signatures in both ALS fibroblasts and mito-ER fractions with a good discrimination (accuracy > 62.5% for metabolomics and >80% for lipidomics). These findings especially highlighted an increase in nucleotides and nucleosides in ALS samples, confirmed by univariate analysis, thus suggesting DNA oxidative damage. We also observed a significant higher mitochondrial oxygen consumption in ALS fibroblasts, with no difference in baseline oxygen consumption rate between ALS and controls cells. Preliminary analysis of mtDNA sequencing did not show significant difference between ALS and controls. Metabolic pathway analysis of the top 15 discriminant metabolites (VIP score > 1.5) suggested disturbance of purine and pyrimidine metabolisms, energy metabolism and glutathione metabolism. It is interesting to note that lipidomics signature of ALS mito-ER fibroblast fraction included phosphatidylcholine PC(36:4p) previously described as a biomarker in CSF of ALS patients and in brain of ALS mice. It has been suggested that this phosphatidylcholine may induce a higher metabolic activity of phospholipase A2 (PLA2), resulting in an increased production of lipid mediators, such as eicosanoids that promote inflammation and are generally considered to play a role in the pathophysiology of ALS. To our knowledge, this is the first analysis combining metabolomics and lipidomics in fibroblasts from sporadic ALS patients. Combination of omics and analysis of isolated mitochondria improved the understanding of some pathophysiological ways and of energetic metabolism disturbance. These data open

perspective of functional, genomic and transcriptomic approaches focused on metabolites identified in this study and help to better understand mechanisms of the disease.

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B38 Upregulation of miR-146a in ALS mouse cortical astrocytes decrease their reactivity and prevents miR-155 transfer into exosomes

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ALS is a neurodegenerative fatal disease of unknown cause. The absence of specific targets has hindered the progress of effective therapeutic strategies. Glial cells, exosomes and miRNAs are accepted as important players in ALS onset, progression and dissemination. Actually, exosomes derived from mSOD1 motor neurons recapitulate microRNA(miR)-124 upregulation and cause microglia activation with mixed phenotypes [1]. We identified low levels of GFAP, upregulated astrocytic S100B protein and connexin 43 (Cx43), and miR-146a, miR-155, miR-124, miR-21 and miR-125b in the spinal cord of symptomatic mSOD1 mice [2]. Intriguingly, astrocytes from the cortex with a neurotoxic profile and elevated S100B and Cx43 levels, showed reduced miR-146a (unpublished data). Since miR-146a is a negative-feedback regulator of the astrocyte-mediated inflammatory process [3], we assessed if the transfection of cortical astrocytes with pre-miR-146a counteracted their aberrant profile. Both mSOD1 and wt astrocytes were isolated from 7-day postnatal mouse brain cortices and cultured for 13 days. Overexpression of miR-146a for 12h led to 8-fold increase in mSOD1 and 2-fold in wt astrocytes ($p<0.05$). Cells were incubated for 24h and exosomes were isolated from cell media by differential ultracentrifugation. Elevation of miR-146a levels re-established the wt number of GFAP+ cells ($p<0.05$) and downregulated HMGB1, S100B and Cx-43 gene expression, together with miR-155, toward control levels. Pre-miR-146a also decreased miR-21 and increased gene and vimentin protein expression in both wt and mSOD1 cells. Exosomes from mSOD1 astrocytes treated with pre-miR-146a showed an increased cargo of miR-146a, together with reduced miR-155 and miR-21, thus recapitulating their donor cells ($p<0.05$). Taken together, we could show that miR-146a reduces the inflammatory response in mSOD1 cortical astrocytes, while lowering miRNA-inflammatory associated exosomal cargo and their propagation to neighboring cells. Our data open new therapeutic strategies for ALS, inasmuch because increased GFAP and vimentin in astrocytes promote axon growth [4] and contribute to astrocyte movement and function. Funded by SCML [ELA-2015-002 (DB) and Research Fellowship (MB)] and FCT [SFRH/BD/102718/2014 (CG), SFRH/BPD/76590/2011 (ARV), and Pest-UID/DTP/04138/2013 (iMed.ULisboa) [1] Pinto et al Front Neurosci 2017 [2]Cunha et al. Mol Neurobiol 2017 [3]Iyer et al. PLoS One 2012 [4]Manzhulo et al. Neurosci Lett 2018

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B39 Characterization of aged TBK1 deficient mice

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by a progressive loss of motor neurons. Amongst the most prevalent causes are mutations in SOD1, C9ORF72, TARDBP, FUS, OPTN and the most recently identified ALS gene TBK1. We and others came to the conclusion that TBK1 haploinsufficiency is associated with ALS and frontotemporal dementia (FTD). The aim of this project is to investigate the molecular mechanisms through which TBK1 haploinsufficiency leads to ALS. Impaired autophagy, accumulation of toxic pTDP-43 aggregates and inflammatory deregulation are the main hypothesis for TBK1 linked ALS/ FTD pathogenesis. TBK1 is responsible for phosphorylation of the autophagasome adaptor protein p62 and Optineurin. Reduction of TBK1 at protein and mRNA level might so decrease activation of autophagy adaptors, resulting in decreased autophagy efficiency and accumulation of these protein aggregates in motor neurons. We provide evidence, at the molecular level, for impaired autophagy in TBK1 +/- primary neuronal culture that does not affect motor abilities in the in vivo situation. The presence of p62 aggregates in our culture might be efficiently compensated in the mice by other mechanisms that have to be further investigated.

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B40 A zebrafish model implicates hnRNPK and hnRNPA3 in C9orf72 RNA toxicity

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A hexanucleotide repeat expansion in C9orf72 is the most frequent cause of ALS. Possible pathological mechanisms include a loss-of-function of the C9orf72 protein and two gain-of-functions driven by toxic RNA, sequestering essential proteins (RNA toxicity), and toxic dipeptide repeat proteins (DPR toxicity), respectively. However, the contribution of each mechanism to ALS pathogenesis and especially the role of RNA toxicity is not fully understood. In this study, we use a recently established zebrafish model to identify new modifiers of RNA toxicity and investigate their modifying mechanisms, ultimately aiming to uncover the pathological underpinnings of RNA toxicity. Using this model, we already identified Pur-alpha, a repeat RNA-binding protein, and p62, a key autophagy protein as modifiers. The aim of our study was to investigate whether candidate modifiers selected from a list of C9orf72 repeat RNA-binding proteins had similar effects. This list included splicing factors and transcriptional regulators. Combined micro-injection of candidate modifier mRNA and repeat RNA in 1 to 2 cell stage zebrafish oocytes led to the identification of two new potential modifiers: hnRNPK and hnRNPA3. Overexpression of these heterogeneous nuclear ribonucleoproteins prevented the motor axonopathy induced by the C9orf72 repeat RNA. Furthermore, we observed a decreased hnRNPK expression level in C9orf72 patient fibroblasts. Expression of C9orf72 repeat RNA in zebrafish also induced hnRNPK mislocalization from the nucleus to the cytoplasm. Using hnRNPK deletion constructs, we are currently trying to reveal which hnRNPK domains are essential for the modifying effect on RNA toxicity. Altogether, our data suggest that two hnRNPs, i.e. hnRNPK and hnRNPA3, could play a role in C9orf72 ALS. Further assessment in patient derived material, i.e. iPSCs or post mortem tissue, will be essential to validate their involvement.

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B41 Features of frontotemporal lobar degeneration in the cyclophilin A knock-out mice

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Cyclophilin A, also known as peptidylprolyl cis/trans isomerase A (PPIA) is a multifunctional protein abundantly expressed in the central nervous system. PPIA has been associated with different human diseases, but its role in pathogenesis is still unknown. We first associated PPIA with nervous system degeneration, identifying PPIA as a translational biomarker of ALS with a protective role of the protein in ALS pathology. We recently demonstrated that PPIA is a functional interacting partner of TDP-43 and regulates its nuclear-cytoplasmic trafficking. TDP-43, a nuclear protein involved in RNA processing, is the major component of neuronal cytoplasmic inclusions in frontotemporal lobar degeneration patients (FTLD-TDP). The molecular mechanisms at the basis of TDP-43 pathology have not been elucidated yet. Here we proposed PPIA as a major player of this process. We show that PPIA knock-out (PPIA-/-) mice recapitulate major features of FTLD-TDP, such as aggregation and mislocalization of TDP-43 in the brain. PPIA-/- mice show also atrophy of cortex and hippocampus, brain regions affected by the disease in the patients. In absence of PPIA, mice exhibited an increased disinhibition and an altered social behavior with no memory and motor impairment reminiscent of the human condition. However, unlike FTLD patients, PPIA-/- mice displayed neuronal with no glial activation. In conclusion, our findings indicate that PPIA has an important effect on neuronal survival and its depletion results in FTLD-related deficits. Moreover, PPIA-/- mouse represents a useful animal model to understand the molecular mechanism behind TDP-43 pathology and its involvement in FTLD-TDP.

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B42 Developing vertebrate models to highlight the functional relevance of NEFL in ALS pathogenesis

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Neurofilaments are the major component of motorneurons, and play a key role in their differenciation, in the establishment and maintenance of their perikaria, dendrites and axon bulks and physical strength. The de novo assembly of these tripartite neuronal intermediate filaments requires the presence of Nefl, their light subunit. Despite the implication of Nefl in several motor diseases, as well as its importance in their diagnosis and prognosis, the mechanism underlying remains elusive. A striking number of genes implicated in ALS pathogenesis encode proteins with functions in RNA metabolism. Some of them interact with mRNAs encoding neurofilament proteins, making them a disease relevant target. In order to address the functional role of Nefl in ALS and identify common therapeutic targets, we decided to take advantage of the zebrafish model, which allows large-scale drug screening and in vivo assessment of biological processes using gene overexpression, knock-down or knock out, and fluorescent transgenic lines. Therefore, we are developing models to study in vivo the functions of these RNA-binding proteins, their functional interactions with neurofilaments, as well as the consequences of their disruption. First of all, we identified and characterized the zebrafish homologues for the low molecular weight neurofilament protein (Nefl) and its expression within physiological and ALS conditions. We established that down regulation of a specific Nefl isoform in zebrafish using morpholinos results in a strong and specific ALS-like motor phenotype (motor axon atrophy and paralysis of the fish). In vivo imaging reveals that Nefl is crucial for proper neuronal development, and that disrupting the balance between its two isoforms specifically affects motor axon growth, as one variant has a tendency to aggregate, both in zebrafish in vivo, and in mammalian cell culture. To confirm these observations, we are currently developing stable Nefl zebrafish mutants using the CRISPR/Cas9 genome editing tools. We also assessed by Western Blot the expression of Nefl within ALS zebrafish models, and revealed that TDP43 knock-down leads to a strong decrease in Nefl protein. Establishing these animal models will allow a better understanding of the role of these key actors in ALS pathogenesis, and will provide relevant endpoints for future studies to identify novel therapeutics targets for ALS.

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B43 Inhibition of histone deacetylases improves motor performances and extends survival in a FUS ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is an incurable and fatal neurodegenerative disorder characterized by the loss of motor neurons in the motor cortex, brainstem and spinal cord. Recently, transcriptional dysfunction was proposed as a novel pathogenic mechanism for ALS, in particular because of the malfunctioning of the enzymes that manipulate epigenetic marks. One of these marks is acetylation of lysine residues of histones, which relaxes the chromatin structure and results in transcriptional activation. Histone acetylation levels are controlled by the antagonistic actions of two protein families, the histone acetyltransferases (HATs) and histone deacetylases (HDACs). There is accumulating evidence that a disturbance in the homeostasis of the acetylation system in favor of the HDACs, resulting in decreased histone acetylation and transcription, is involved in ALS pathogenesis. The aim of this study was to investigate the therapeutic potential of HDAC inhibitors in the PrP-hFUS-WT transgenic mouse model for ALS, as we observed histone hypoacetylation in the central nervous system (CNS) of these mice. This model overexpresses human wild-type FUS exclusively in the CNS resulting in severe motor impairment starting at 4 weeks and in death at 9 weeks. This aggressive ALS phenotype is caused by progressive muscle denervation, axon degeneration and ~60% motor neuron loss at end-stage. Treatment with an HDAC inhibitor (HDACi) starting at disease onset restored histone acetylation and increased survival of the FUS ALS mice with 40%. This was accompanied by an improved motor performance. We also observed increased compound muscle action potentials (CMAPs) measured at the gastrocnemius muscle, reflecting an improved functionality of the motor unit. In order to get a better understanding on the pathogenic processes underlying FUS-mediated ALS and the mechanism of the therapeutic effect of the HDAC inhibitor, we performed RNA sequencing on spinal cord tissue. This revealed that PrP-hFUS-WT mice have altered mRNA levels of genes involved in metabolic pathways and that HDACi treatment partially corrected these changes. Notably, the altered levels of metabolic transcripts already appeared at disease onset and correlated with the progressive decline in motor performance. In conclusion, our data suggest that transcriptional changes of metabolic genes are an early event in ALS, and that histone deacetylases are potential therapeutic targets to delay disease progression.

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B44 Targeted Drosophila screen reinforces nucleocytoplasmic transport to DPR pathology in C9orf72-associated ALS/FTLD

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting the upper and lower motor neurons in the motor cortex, brainstem and spinal cord. This leads to a wide variety of symptoms ranging from limb paralysis to losing the ability to speak, eat and ultimately breathe. In 2011 intronic hexanucleotide (GGGGCC) repeat expansions were discovered in chromosome 9 open reading frame 72 (C9orf72) as an important genetic cause of ALS. Until now, three mechanisms for C9orf72 toxicity have been hypothesized: haploinsufficiency, RNA toxicity or dipeptide repeat proteins (DPRs) toxicity. These DPRs are generated by repeat associated non-ATG mediated (RAN) translation and yields five DPRs: GA, GR, PR, PA and GP. We and others have shown that especially arginine-rich DPRs (GR and PR) are toxic. To further investigate this toxicity, we generated Drosophila models expressing DPR specifically in the eye using a GMR driver. Expression of the PR dipeptide yielded a moderate rough-eye phenotype, which we used in a targeted RNAi screen focused on nuclear transport. The aim of this study was to investigate whether toxicity induced by GR DPRs could be influenced by the same modifiers as the ones discovered in the PR screen. Therefore, we generated a Drosophila model specifically expressing GR in the eye and repeated the initial screen. We could nicely recapitulate the same hits as in the PR screen. The most potent modifiers from both screens were members of the nuclear import and export pathway, respectively transportin-1 and exportin-1. In addition, modifiers were identified in the nuclear pore complex, and the Ran-GTP cycle. The discovery of these modifiers further supports the hypothesis that DPR pathology of the arginine-rich proteins in C9orf72 ALS disrupts nucleocytoplasmic transport, and could initiate the ALS disease cascade hallmarked by protein mislocalization and aggregation.

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B45 Acetylation state of RelA modulated by epigenetic drugs prolongs survival and induces a neuroprotective effect on ALS murine model

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that cause degeneration of motor neurons of central nervous system. Defects in histone homeostasis has been recently implicated in the pathogenesis of neurodegenerative diseases, including ALS. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) catalyze the acetylation and deacetylation, respectively, of histone proteins. HATs and HDACs use as substrates also transcription factors, such as nuclear factor (NF) kB. Transcriptional dysregulation occurs in human sporadic ALS and in the SOD1(G93A) mouse model. Five DNA-binding proteins can compose the NF-kB complex. The NF-kB dimer p50/RelA has a dual, neuroprotective or neurotoxic effect depending on its acetylation state. Our aim is to test if the treatment with epigenetic drugs modulates the acetylation of RelA in the spinal cord of SOD1(G93A) mice, slowing down the disease progression of ALS. In order to promote a proper acetylation of NF-kB, a combination of the HDAC 1-3 inhibitor MS-275 and the sirtuin 1 activator Resveratrol were administered intraperitoneally every day in SOD1(G93A) mice at beginning of 50 day of life, until the death of the animals. Behavioral tests showed a significant improvement of motor performance ($p<0.05$) of treated group versus control group. Furthermore a delay of pathological onset and an increase of survival rate ($p<0.05$) were detected in the treated group compare to the untreated once. Moreover, the epigenetic treatment elicited a neuroprotective effect on the lumbar spinal cord motoneurons of treated group compare to control group, accompanied by increased levels of protein products of NF-kB-target genes, Bcl-xL and brain-derived neurotrophic factor. Our study reveals that the combined epigenetic drugs delay the degenerative process that occurs in ALS, representing a future promising therapy for this disease.

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B46 Impaired stress granule dynamics in motor neurons from a novel mouse model of TDP-43-associated ALS

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Background The mechanisms underlying the preferential loss of motor neurons in ALS are still poorly understood. The vulnerability of these cells may be due, in part, to a defective response to conditions such as oxidative stress. Mutations in TDP-43, the pathological hallmark protein of ALS, can impair the normal assembly of stress granules. Disrupted stress granule dynamics might be central to the pathogenic mechanism in ALS. We have previously developed a novel bacterial artificial chromosome (BAC) transgenic mouse expressing human TDP-43 (M337V) at low levels, which develop distinct motor deficits (rotarod and grip strength), survival deficits in the second year and pathological phenotypes (reduced axonal transport, NMJ deficits) recapitulating some key features of ALS. Methods Primary motor neurons (MNs) were generated from E13.5 lumbar spinal cord from non-transgenic (NTg), TDP-43WT/- and TDP-43M337V/- embryos. Mouse ESCs (non-transgenic or expressing the TDP-43 BAC constructs) were expanded as embryoid bodies and differentiated into motor neurons. Cells were stressed with 0.5mM sodium arsenite, then immunostained for total TDP-43, human TDP-43 and stress granule markers TIA-1, PABP1 and G3BP. Survival assays were also performed to assess the effect of mutant TDP-43 and oxidative stress on motor neuron survival. Results TDP-43 was predominantly cytoplasmic in TDP-43M337V/- primary motor neurons, compared to TDP-43WT/- and NTg controls. Concurrent with cytoplasmic mislocalisation of TDP-43, TDP-43M337V/- MNs (primary or ESC-derived) displayed a reduction in the proportion of cells containing stress granules and a decrease in stress granule size, in response to oxidative stress. We also observed reduced stress granule recruitment of mutant TDP-43 and significantly reduced survival in response to increasing exposure to oxidative stress. Conclusions Our data suggest that impaired stress responses may underlie the link between mutant TDP-43 and selective motor neuron vulnerability in our mouse model of M337V-associated ALS. With the Oxford Target Discovery Institute we have used high-throughput screening (HTS) of FDA approved compounds to identify drugs which might restore the stress granule response in motor neurons, which can then be validated through detailed analysis of phenotypic and transcriptional changes in primary MNs from our TDP-43 BAC transgenic mice, and iPSC-derived motor and cortical neurons from ALS patients carrying TDP-4

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B47 Identification and validation of nuclear and cytoplasmic TDP-43 protein binding partners in a mouse model of ALS

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The only marketed drug, Riluzole, prolongs life expectancy in Amyotrophic Lateral Sclerosis (ALS) patients for up to three months. Given the poor prognosis for this disease, accelerating both research-based and pre-clinical studies is critical for ALS drug development. Advancement of effective therapeutic strategies for ALS has largely been hampered due to i) lack of in vitro models that are robust and high throughput and (ii) our limited understanding of mechanisms underlying the disease. Current approaches such as transient transfection or patient-derived motor neurons to model ALS are very informative though highly laborious for primary screening of novel candidate compounds. Here, we present a robust high throughput, high content screening method to identify and quantify pathological features in an inducible human neuroblastoma SHSY5Y cell line expressing wild-type TDP-43 (GFP-tagged). By delineating nuclear vs cytoplasmic TDP-43 expression combined with high-content analyses, we demonstrate several pathological features observed in sporadic and familial TDP-43 patients, including TDP-43 hyperphosphorylation, increased cytoplasmic TDP-43 mislocalisation as well as altered autophagy (using P62 as a marker) and neurotoxicity. The addition of osmotic and oxidative stressors further exacerbates this phenotype. Further, we use this approach, in combination with mass spectroscopy to identify and validate cytoplasmic TDP-43 protein binding partners which drive pathological aggregation. Nuclear and cytoplasmic lysates from a transgenic mouse model of ALS were extracted and TDP-43 was co-immunoprecipitated. Subsequently, we demonstrate the effects of TDP-43 associated pathology upon knockdown of a selection of prominent TDP-43 protein binding partners in mouse brain slices derived from ALS mouse models. We anticipate that outcomes from these studies will advance our understanding of TDP-43 disease mechanisms which can be extended for other proteinopathies leading to neurodegeneration.

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B48 Chemotherapeutic agent 5-Fluorouracil improves performance of mutant SOD1 mouse model of ALS

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Amyotrophic lateral sclerosis (ALS) is a lethal motor neuron disease with only a single widely used but only marginally effective drug treatment. In the search for drug candidates for ALS, we studied the effect in SOD1G93A model of ALS of known stem cell mobilizing agents (treatment) and antimetabolite 5-fluorouracil (5-FU) (anti-treatment). Surprisingly, it was found that anti-cancer drug 5-FU increases lifespan, delays disease onset and improves motor performance in ALS mice. Although we were not able to demonstrate the mechanistic basis of the beneficial 5-FU action our findings suggest that 5-FU or similar drugs could be possible drug candidates for repurposing to the treatment of motor neuron disease. The studies concentrating on target tissues and cell types, as well as molecular mechanistic basis of these substances on ALS models are warranted.

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B49 Effects of gamma-carbolines on pathology caused by expression of C-terminally truncated human FUS in the nervous system of transgenic mice

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Results of previous studies suggested that certain gamma-carbolines might act as neuroprotectors in the nervous system affected by neurodegenerative processes. We used a model of severe early onset and fatal neurodegeneration caused by expression of the C-terminally truncated human FUS (heterozygous FUS 1-359 transgenic mice) to assess the effect of chronic administration of gamma-carboline Dimebon and its derivates DF302 and DF402 on the disease onset and duration, and animal lifespan. Of these three compounds, DF402 demonstrated the most marked effect, particularly on the disease duration, which led to significant increase of animal lifespan. More detailed characterisation of the effect of DF402 on animal nervous system at presymtomatic stage is required for better understanding of the mechanism of the drug action. We employed RNAseq to compare transcriptomes of the thoracic spinal cords of 80-day old presymptomatic DF402-treated and untreated FUS 1-359 transgenic mice and wild type mice of the same age. From 127 genes upregulated in FUS-TG 1-359 mice, 29 were found to be significantly (padj<0.05) downregulated in DF402-treated mice, returning their expression to the level in wild type mice. From 94 genes downregulated in FUS-TG 1-359 mice, 25 were found to be significantly (padj<0.05) upregulated in DF402-treated mice, again returning their expression to the level in wild type mice. One of the challenges for further studies is to single-out within the same age group those animals that started developing the very first signs of motor dysfunction. To achieve this we used Catwalk gate analysis system. Testing of FUS 1-359 and wild type littermates started at the age of 30 days and continued on the daily basis until the symptoms were visible. After classification of footprints using the CatWalk XT software data were exported for external analysis using R algorithm to perform multi-dimension scaling analysis. Data from 499 mice and 308 parameters were analysed. We were able to select a set of parameters distinguishing between transgenic and wild type mice long before clinical signs of neurological pathology become visible. This study was supported by Motor Neurone Disease Association Grant 822-791 to VLB. Bioresource Collection of IPAC RAS (No. 0090-2017-0016) facilities were used to maintain animals for CatWalk data collection using equipment of the Centre for Collective Use IPAC RAS.

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B50 The effect of intermediate polyQ expansions in Ataxin-2 on TDP-43 pathology in vivo

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Ataxin-2 (ATXN2) is an intracellular protein with diverse roles, including RNA metabolism, and a risk factor for ALS. Intermediate-length polyglutamine expansions (polyQ; from 22 to 27-33 Qs (CAG)) in ATXN2 are associated with the disease. Further, ATXN2 has been reported to enhance TDP-43 and FUS toxicity, as well as to synergize with the effects of C9ORF72 loss-of-function. This suggests that ATXN2 has a general role in ALS pathology and that therapeutic interventions targeting ATXN2 could be beneficial for a large group of patients. Indeed, lowering of ATXN2 levels was recently shown to extend lifespan and reduce pathology in an acute TDP-43 mouse model. However, the mechanisms through which ATXN2 repeat expansions contribute to disease development remain largely unknown. Therefore, we aim to study the contribution of ATXN2, and its repeat expansions, to ALS pathogenesis. We have engineered BAC transgenic mice carrying the entire human ATXN2 locus. Since mice do not have polyQ repeats in Atxn2, we generated a control human ATXN2-CAG22 mouse line and an ALS-associated human ATXN2-CAG33 mouse line. These mice were crossed with a novel TDP-43(M337V) mouse model that displays ALS pathological features and a progressive age-dependent motor phenotype. Preliminary data reveal changes in grip strength in ATXN2-CAG/TDP-43 (M337V) mice and unexpected changes in bodyweight. These new mouse models will help to dissect the pathological mechanisms underlying ATXN2 repeat expansions in ALS and to identify therapeutic strategies counteracting these mechanisms.

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B51 Conditional deletion of Id2 in oligodendrocyte progenitor cells does not ameliorate disease outcome in SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with selective upper and lower motor neuron degeneration. Patients suffer from progressive muscle atrophy and will typically die within 2 to 5 years after disease onset as no effective treatment is currently available. Although traditionally viewed as a motor neuron disease, damage developed within non-neuronal supporting cells is crucial to motor neuron dysfunction in ALS. Oligodendrocytes are such contributing non-neuronal cells, as they degenerate during disease and are replaced by newly formed oligodendrocytes. However, in both ALS patients and mutant SOD1 mice, these newly formed oligodendrocytes are immature and dysfunctional, as they insufficiently generate myelin basic protein (MBP) and monocarboxylate transporter 1 (MCT-1). Consequently motor neurons lose an important source of structural and trophic support. Therefore, strategies to improve differentiation of oligodendrocyte progenitor cells (OPCs) towards mature oligodendrocytes, could be of therapeutic interest in ALS. Here we report that oligodendrocytic ablation of Inhibitor of DNA binding 2 (Id2), a negative master modulator of oligodendrocyte differentiation, fails to alleviate oligodendrocyte dysfunction or alter disease outcome in a murine model of ALS. Our data suggest that this inhibitor is not a suitable target for intervention in ALS.

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B52 Bespoke mouse models of ALS

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Our group is aiming to generate new and bespoke mouse models for neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS; motor neuron disease), Alzheimer's disease, and Parkinson's disease. Very few treatments, and no cures, exist for these diseases that are only increasing in prevalence in our ageing population, while many key biological questions remain unanswered. Modelling late onset neurodegenerative disease is challenging, and improved models are needed to more faithfully recapitulate human pathology. One key long-term focus of the lab is to genetically engineer, and phenotype, new models of neurodegeneration via genomic humanisation of the mouse to answer key questions surrounding mechanisms of action, and to provide physiologically relevant models to test future therapeutics. Here we present ongoing work on humanising two genes, C9ORF72 and FUS, and their respective mutations that cause ALS – a fatal disorder that results in progressive degeneration of motor neurons. A hexanucleotide repeat expansion in the C9ORF72 gene represents the most common genetic cause of ALS and frontotemporal dementia. Our project to humanise this gene at the endogenous locus, with and without repeats, is in the initial phase, and we outline our BAC recombineering progress here. We also present data on a new mouse model generated by Anny Devoy and Elizabeth Fisher at UCL, harbouring a partially humanised FUS gene plus human pathogenic mutation causative for ALS. The mutant FUS-delta14 protein mislocalises to the cytoplasm and leads to progressive lower motor neuron loss starting mid-life. Finally, we present some preliminary data from our new project to humanise GJA1 and GJA1P1 genes, which have recently been found to have a potential role in the pathological mechanisms of neurodegenerative diseases like ALS. We have strong collaborative ties with neuroscience and neurodegeneration experts and clinicians at the UCL Institute of Neurology/National Hospital for Neurology and Neurosurgery, which we combine with world class mouse genetics resources here at Harwell to answer key questions in neurodegeneration.

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B53 A feedback loop between dipeptide repeat protein, TDP-43 and karyopherin-α mediates C9ORF72-related neurodegeneration

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TDP-43 accumulation is a major hallmark of amyotrophic lateral sclerosis and frontotemporal dementia, including the most common genetic cause, G4C2 hexanucleotide repeat expansion in C9ORF72 (C9ALS/FTD). The role of TDP-43 dysfunction in C9ALS/FTD, however, remains elusive. We found G4C2-derived dipeptide repeat protein (DPR) but not G4C2-RNA accumulation caused TDP-43 proteinopathy that triggered onset and progression of disease in Drosophila models of C9ALS/FTD. Timing and extent of TDP-43 dysfunction was dependent on levels and identity of DPRs produced, with poly-GR causing early and poly-GA/poly-GP causing late onset of disease. Accumulating cytosolic, but not insoluble aggregated or mutated TDP-43, caused karyopherin-α2/4 pathology, increased levels of DPRs and enhanced G4C2-related toxicity. Comparable karyopherin-α4 pathology was observed in sporadic FTD and C9ALS/FTD patient tissue, irrespective of TDP-43 or DPR aggregates. These findings identify a vicious feedback cycle for DPR-mediated TDP-43 and subsequent karyopherin-α pathology, which becomes self-sufficient of the initiating trigger to cause neurodegeneration.

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B54 Mutations in TARDBP show axonal transport defects in induced pluripotent stem cell-derived motor neurons

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TAR DNA binding protein 43 kDa (TDP-43) is a major component of pathological inclusions in sporadic and familial amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U). ALS patients suffer from progressive degeneration of motor neurons, while FTLD is characterised by the progressive degeneration of cortical neurons in the frontal and temporal lobe. Moreover, mutations in the gene encoding TDP-43 have been directly linked to ALS. The aim of this study was to investigate whether mutant TDP-43 affects transport processes along the axons, which is important for the normal function of motor neurons. We generated and characterized human induced pluripotent stem cells (hiPSCs) from ALS patients with different TARDBP mutations, as well as from healthy controls. The hiPSC lines were subsequently differentiated into motor neurons in order to study axonal transport. We therefore labelled mitochondria in motor neurons with MitoTracker-RED. Subsequently, mitochondrial movement along the processes of the motor neurons was registered by live cell imaging, and the number of stationary and moving mitochondria was determined. Compared to control lines, the average number of moving mitochondria was significantly lower in motor neurons derived from patients with a TARDBP mutations. Furthermore, pharmacological inhibition of histone deacetylase 6 (HDAC6) increases Î±-tubulin acetylation, and restores the axonal transport defects in patient-derived MNs. Taken all together, our results clearly show that mutations in TARDBP cause impairments in axonal mitochondrial transport in hiPSC-derived motor neurons and inhibition of HDAC6 results in axonal transport restoration.

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B55 Design of an inducible system to test the toxicity of dipeptide repeats in C9orf72 iPSC-derived motor neurons from ALS patients

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Background An intronic repeat expansion (GGGGCC)n in C9ORF72 gene is the commonest cause of amyotrophic lateral sclerosis (ALS) identified to date. The mechanisms proposed to explain neuronal degeneration induced by (GGGGCC) n are loss of function of C9ORF72, transcription of the expansion in toxic RNAs, translation of both sense and antisense transcripts, generating five different dipeptide repeats (DPRs). Aim This project aims to analyse the contribution of DPRs to the development of ALS phenotypes in induced pluripotent stem cells (iPSCs)-derived motor neurons (MNs). Although there is evidence for the toxicity of some DPRs, few studies have examined the role of DPRs when they are expressed in combination. Methods We generated a lentiviral backbone carrying a doxycycline inducible system (dox-ON) which controls the expression of GFP or DPRs. The sequences of three DPRs – poly(GA)50, poly(GR)50, poly(PR)50 – were optimised to avoid the production of RNA foci, and each DPR was fused to a different tag. We differentiated MNs from patient iPSCs carrying the (GGGGCC)n repeat, healthy cells, and isogenic lines where the expansion has been excised by CRISPR/Cas9 system. Healthy and edited lines were transduced with lentivirus carrying the optimised DPR sequences and their phenotypes were compared to the patient lines. Results Immunoblotting and live fluorescence in HEK293T cells transfected with the dox-ON lentiviral backbone showed efficient expression of GFP after the addition of doxycycline. The cells expressed GFP after 24 hours of treatment, and removal of doxycycline inactivated the expression. Control iPSC lines were transduced with dox-ON lentivirus and various concentrations of doxycycline were tested to determine the optimal one for inducing the expression of GFP. The sequences expressing (GA)50-cMyc, (GR)50-flag and (PR)50-HA replaced GFP, and iPSCs were transduced and analysed to test if the conditions used previously led to efficient transduction and induction. Conclusions The dox-ON system developed in this project allows fine control of the expression of each DPR, a feature conferred by the sensitivity of the system to low doses of doxycycline. In addition, the optimised sequences of DPRs avoid the formation of RNA foci, and allow the expression of one protein at a time. The whole system allows to test different concentrations and combinations of DPRs, as well as to examine if the MNs can recover in response to switching off DPR production.

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B56 C9orf72 iPS-derived motor neurons have altered cytosolic and mitochondrial calcium buffering

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Hexanucleotide expansions in the C9orf72 are the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). To better understand the direct contribution of the hexanucleotide repeats to cellular phenotypes, we used C9orf72 patient motor neurons derived from induced pluripotent stem cells (iPSCs) and an isogenic iPSC line where the expansions were successfully removed by CRISPR/Cas9 and homology directed recombination. The results were compared to iPS-derived MNs from TDP-43 patients. In C9orf72 iPS-derived MNs we found significantly higher Ca2+ release when neurons were depolarized. Clearance of calcium from the cytosol was significantly delayed in C9orf72 MNs after stimulation with KCl and glutamate, which correlated with low levels of the calcium-buffering protein calbindin and increased cell death. The levels of calcium binding proteins in TDP-43 MNs were not significantly different. An impairment was also observed in the calcium-buffering capacity of mitochondria in C9orf72 MNs, where low levels of the mitochondrial calcium uniporter (MCU) were detected and correlated with reduced uptake of Ca2+ from the cytosol compared to healthy controls and corrected MNs. The mitochondrial potential was reduced in C9orf72 MNs, while the TDP-43 M337V and I383T MNs did not show differences when compared to healthy controls. To investigate the functional consequences of signalling deficiencies, C9orf72 MNs were co-cultured with muscle cells in microfluidic chambers and we found that the area occupied by the NMJs formed between C9 MNs and muscle cells was significantly smaller than the area of healthy and edited MNs. However, the number of NMJs formed between axons and muscle cells was not different between C9orf72 MNs and edited or control MNs. This study shows a deficiency in the calcium-buffering capacity of C9orf72 MNs, partially due to inefficient mitochondrial Ca2+ uptake and low levels of calbindin and indicates that the mitochondria plays an important role in C9orf72 pathogenicity.

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B57 Using iPSC-derived motor neurons to explore protein misaccumulation and cellular dysfunction in motor neuron disease

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Motor neurons (MNs) differentiated from induced pluripotent stem cells (iPSCs) derived from MND patients with TARDBP mutations are used to develop a model of TDP-43 pathology for identification of potential therapeutic targets. Immunocytochemistry is used to assess disease phenotype in a high-throughput setting. CRISPR-Cas9 was used to correct the mutations to produce isogenic control lines in order to provide a direct comparison between mutant and wild-type iPSCs with an identical genetic background for assessment of potential targets. MNs with TARDBP mutations recapitulate aspects of MND pathology, including increased TDP-43 expression, aggregation and phosphorylation. M337V mutant MNs also show reduced ATP levels, reduced mitochondrial membrane potential and decreased ER-mitochondrial contact compared with controls, indicating mitochondrial dysfunction. Lentiviral delivery of an shRNA panel will then be used to knockdown protein pathways implicated in disease progression, and resulting phenotypic changes will be monitored.

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B58 Development of a human stem cell-derived neuromuscular in vitro system

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Spinal motor axons span long distances through the body to connect to target muscles via specialized synapses termed neuromuscular junctions (NMJs). In ALS, deterioration of NMJs represents the earliest visible sign of degeneration. The pathology then progresses proximally towards the soma, in a dying-back fashion. In fact, denervation occurs well before loss of motor neuron somas or clinical symptoms. To study the neuromuscular connection in ALS and health, we previously generated NMJ-like structures by culturing mouse embryonic stem cell-derived motor neurons with mouse myofibers differentiated from primary satellite cells in microfluidic devices (Mills, 2018). Using a compartmentalized microfluidic device, spatial separation and fluidic isolation of neuronal somas from their NMJs with myofibers enables precise control and manipulation of the cellular microenvironments. We are now developing an entirely human stem cell-derived NMJ model system. The system is designed to become a valuable tool for basic and translational research with motor neurons and myofibers specified from control, as well as ALS patientâ€'derived, induced pluripotent stem cell (iPSC) lines. Here we differentiate and culture human iPSC-derived motor neurons in microfluidic devices, where motor axons successfully traverse microgrooves into the adjacent compartment with myofibers. Furthermore, we evaluate two distinct approaches to differentiate human iPSCs to skeletal muscle for their efficiency in giving rise to myogenic cells, mature myofibers and satellite cells. The first differentiation protocol aims to recapitulate key signaling events during in vivo myogenesis. The second is a commercially available differentiation kit, generated by high-throughput screening of media and factors for their myogenic potential. Ultimately, the development of a human in vitro NMJ model opens up possibilities for screening of potential therapeutic targets aimed towards stabilizing the NMJ in ALS.

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B59 Motor neuron differentiation of iPSCs from peripheral blood of a TARDBP mutated ALS patient

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Difficulty of translation from preclinical to clinical settings represents the main limitation in deciphering pathomechanisms of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), and this is primarily due to the shortage of adequate pre-clinical models. The induced pluripotent stem cells (iPSCs) represent a breakthrough in stem cell field. These cells may be generated using several delivery systems and certainly present numerous advantages compared to other pre-clinical approaches, allowing the generation of patient specific models able to reproduce the disease phenotype. In this study, motor neurons (MNs) were differentiated from peripheral blood mononuclear cells-derived iPSCs of an ALS patient carrying the mutant TDP-43 p.A382T and of an healthy donor. Peripheral blood cells present advantages compared to fibroblasts as they do not require time consuming in vitro cultures and consequent risk of genomic alterations. Furthermore, peripheral blood collection is less invasive than skin biopsy. iPSCs were generated using integration-free Sendai virus reprogramming factors and emerged colonies were individually picked and expanded for six passages before characterization, differentiation and freezing. Pluripotency of iPSCs was investigated by expression of specific markers (Sox2, Oct3/4, Nanog, Alkaline Phosphatase, SSEA4 and Tra-1-60) and spontaneous differentiation into the three primary germ layers endoderm, ectoderm and mesoderm. No differences were observed between the mutated patient and the control iPSCs concerning their pluripotency. iPSCs were then differentiated into MNs and comparable expression of axonal neurofilament SMI 312 and MN specific HB9 markers was observed. We finally investigated whether MNs differentiated from the TARDBP mutated iPSCs, without stressors, displayed key aspects of ALS such as TDP-43 protein mislocation and aggregation. We found that most of the cells displayed a nuclear localization of TDP-43 protein in both patient and healthy control samples. In conclusion, we demonstrated for the first time that motor neurons can be successfully obtained from peripheral blood derived iPSCs carrying the TARDBP p.A382T mutation with no TDP-43 aggregation or abnormal cytoplasmic distribution.

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B60 Neuronal excitability of ALS patient-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive loss of upper and lower motor neurons (MNs). Until now no effective treatment is available. Underlying mechanisms leading to MN death are still largely unknown, but altered neuronal excitability has been hypothesized to contribute to neurodegenerative pathways in ALS. The current study aims at analyzing whether neuronal excitability is changed in MNs from ALS patients harboring C9Orf72, TARDBP and superoxide dismutase 1 (SOD1) mutations. To this end, induced pluripotent stem cells (iPSCs) from ALS patients with C9Orf72, TARDBP and SOD1 mutations were differentiated into MNs. iPSC-derived MNs from two healthy patients served as controls. In addition, MNs derived from a gene-corrected isogenic control iPSC line from an ALS patient carrying a SOD1A4V mutation were also included. Neuronal excitability was studied by performing whole-cell patch clamp recordings on iPSC-derived MNs. MNs obtained from controls and mutant C9Orf72, TARDBP and SOD1 ALS patients displayed appropriate functional properties i.e. the ability to fire action potentials and the presence of voltage-gated Na+ and K+ channels. Analysis of the frequency of action potentials revealed no difference in excitability between control and C9Orf72 MNs. In contrast, MNs from TARDBP ALS patients were less excitable than control MNs. Also, SOD1 MNs show a tendency towards a hypo-excitable phenotype compared to the isogenic control MNs. These findings demonstrate that excitability of ALS patient-derived MNs may change in a genotype-dependent manner.

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B61 Ryanodine receptor and IP3 receptor role in the ER-mitochondria-calcium-cycle of IPSC derived ALS motor neurons

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Background: Dysregulation of the Endoplasmic Reticulum-Mitochondria-Calcium-Cycle (ERMCC) appears to play a major role in the pathophysiology of ALS. Both Ryanodine receptor (RyR) and Inositol-1,4,5 receptor (IP3R) induce calcium efflux from the ER. As ER calcium depletion is a central aspect of the complex pathogenesis in ALS, disturbances in expression and function of RyR and IP3R are likely to be involved in the perturbation of the ERMCC. Objectives: We aim to reveal the role of RyR and IP3R on calcium homeostasis. Therefore, we aimed to investigate (i) location and distribution (ii) protein expression level under basal condition and after modulation and (iii) if modulation can rescue MNs from kainate-induced excitotoxicity. Furthermore, we aim to analyze and compare (iv) neurite length and (v) branch points of MNs carrying different ALS-related mutations. Methods: Induced pluripotent stem cells-derived motor neurons from patients carrying ALS mutations SOD1R115Gor C9orf72-HRE and healthy controls were used for immunofluorescent staining and live cell imaging. Neurite length and branch points were compared under different conditions over 18 days in automated microscopy analyses. Neuronal cell survival was assessed after RyR- and IP3R treatment with or without kainate for 24 hours. Results: Neurite length development and branch point counts were significantly reduced in MNs carrying mutations versus control MNs. Mutant SOD1 carrying MNs were resistant to cessation of media change. Preliminary data indicate that the presence of mutant SOD1 increases RyR and IP3R expression suggestive of ER calcium depletion and UPR activation. Conclusion: ALS mutations affect neurite morphology at the basal level and increase RyR and IP3R expression in motoneurons carrying human disease causing mutations. Further studies need to be carried out to test the therapeutic potential of RYR and IP3R modulation. Acknowledgment: This research is supported by a grant of the European Font for Regional Development (EFRE Program of the Thüringer Aufbaubank) and the Dt. Gesellschaft für Muskelkranke (DGM).

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Seminar Room A:

D01 The effect of a healthcare training programme on clinical usage of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

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Background Approximately 50% of people with ALS experience changes in cognition and/or behaviour. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) consists of a brief assessment of the cognitive functions affected in ALS and a screen for behavioural symptoms. A masterclass in how to administer the ECAS was made available to healthcare professionals involved in ALS care across the UK in 2017. Aim To evaluate the effect of implementing an ECAS training programme for healthcare professionals and the subsequent clinical impact of its use. Method 1. An ECAS masterclass was run in 9 locations throughout the UK between Mar-Sep 2017. 2. An online survey of cognitive and behavioural screening practices in ALS care centres and care teams was carried out at Time 1 (Feb-Jun 2017), prior to implementation of the ECAS masterclass, and at Time 2 (Febâ€"Jun 2018), post-masterclass. 3. The clinical impact of the ECAS was assessed using comparative case studies. Six healthcare settings have been purposively selected. Semi-structured interviews are being conducted with health professionals, patients and carers. Data are analysed thematically. Results Health care professionals (N=245) were trained on the programme. At Time 1 survey data was collected from 26 sites (19 ALS care centres & 7 community/hospice-based teams). In 24 of the 26 sites surveyed, some patients received cognitive and or behavioural assessment, however, routine screening was not common practice across sites. Typically, the clinical neuropsychologist or the nurse undertook the assessment and, on occasion, an OT or a neurologist, usually in a clinic setting. Collection of Time 2 survey data is currently underway. Furthermore, findings from the comparative case studies exploring clinical impact will be reported. Discussion We expect the survey data to inform the ALS care community of disparities in provision of cognitive and behavioural screening and to highlight the benefits of training, including where further training and support may be required. This is the first study to explore the benefits of using the ECAS from the perspectives of healthcare professionals, patients and carers. Comparing and contrasting across different types of healthcare settings will highlight how different teams have managed to forge a pathway to screening within their local context and the resources required to realise the potential impact.

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D02 Usability of eyetracking computer systems and impact on psychological wellbeing in patients with advanced amyotrophic lateral sclerosis

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Restrictions in communicative abilities are well known in patients with Amyotrophic Lateral Sclerosis (ALS), but only few approaches in terms of evaluation of supportive technologies have been made. We aimed to assess the use and perceived usability of eye-tracking computer devices (ETCS) and (ii) the quality of life (QoL) and psychological wellbeing in patients with ALS-induced locked-in-state and their next of kin in a fully unbiased, direct manner, using ETCS . ETCS enable active communication and social participation in the quadriplegic and anarthric disease state. Therefore, ETCS-based versions of widely used psychosocial questionnaires (ADI-12, SeiQoL-DW, WHO-5) as well as structured questions on communicative functioning and ETCS-usage were developed to assess ALS patients, their next of kin and professional caregivers. Eleven patients (ALS-FRS-R: 5.3±5.9; ALS duration: 6.5±3.8y, range 1-12; 82% invasively ventilated), 9 next of kin and 10 professional caregivers could be assessed. Patients reported a mean use of their personal ETCS of 9.1 hours per day (range: 0.5-16), with a high user satisfaction, preservation of communicative abilities and subjective indispensability of the ETCS. Patients reported good QoL which appeared to be at the cost of the QoL of their next of kin. Next of kin rated their own or patients' QoL similarly, but they identified different areas as important as compared with patients. Our results are of importance for the discussion of end-of-life-decisions and the evaluation of the patient's presumed wishes as well as for psychosocial interventions. Our results strengthen the evidence that preserved mental autonomy influences psychological wellbeing in ALS and might even modify disease course and end-of-life-decisions in ALS. Funding: We thank all subjects who participated in this study. We deeply appreciate the help of the patient network "ALS mobil e.V.". The study was funded in part by a grant of the German ministry for education and research (BMBF, 16SV5843), the Roland Ernst Stiftung Saxony, Germany, and by the „Innovationsausschusses des Gemeinsamen Bundesausschuss", Germany (FKZ 01VSF16026).

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D03 The Edinburgh Cognitive and Behavioural ALS Screen: Relationship to age, education, IQ and the Addenbrooke's Cognitive Examination-III

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The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was developed to assess cognitive and behavioural changes common in Amyotrophic Lateral Sclerosis and other diseases affecting motor functions. It focuses on domains typically affected by the frontotemporal syndrome (executive and language functions, fluency and behaviour), but assesses also memory and visuospatial functions. Objectives: A. To investigate the relationship between the ECAS and the Addenbrooke's Cognitive Examination (ACE-III). B. To investigate the effects of age, education, and IQ on the ECAS and create appropriate cut-off scores to determine abnormality. Methods: A: 57 healthy participants (aged 35-80) were assessed with the ECAS, the Wechsler Abbreviated Scale of Intelligence (WASI-II), and the ACE-III. B: 80 healthy participants (aged 51-80) were divided into four groups according to age and education and were tested with the ECAS and the WASI-II. Results: The ECAS and the ACE-III have a strong convergent validity with a significant correlation. Regression analysis revealed that IQ, followed by age, were the strongest predictors of the total ECAS score. IQ predicted 24% of the ECAS and 46% of the ACE-III variance. Education was not a significant predictor over and above IQ for both the ECAS and the ACE-III. Abnormality cut-off scores adjusted for age and education are presented. Conclusions: The ECAS shows good convergent validity with the ACE-III, but is less influenced by intelligence and presents less ceiling effects. The inclusion of an executive function assessment and behavioural interview in the ECAS makes it particularly useful for the assessment of frontal lobe disorders.

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D04 Characterising psychological trauma resulting from being given a diagnosis of Motor Neuron Disease (MND)

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Background: Motor neuron disease (MND), also known as Amyotrophic Lateral Sclerosis (ALS), is the most common neurodegenerative disorder of the motor system in adults. The psychological impact of being given a diagnosis of MND has been poorly studied, with a scarcity of research exploring the symptoms of psychological trauma directly resulting from being diagnosed with MND, and the implications of these symptoms for patients. Aims: The purpose of this study was to gain insight into the prevalence and phenotype of psychological trauma symptoms related to the event of being given a diagnosis of MND in two countries. Methods: A case series study was carried out at the Motor Nerve Clinic at King's College Hospital in London (UK) and at the HD Respiratory Rehabilitation of Fondazione Don Carlo Gnocchi in Milan (Italy). Adult patients who were given a diagnosis of MND within the previous one to four months, were recruited. After collecting demographic and clinical information, participants completed the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), the PTSD Checklist for DSM-5 (PCL-5), The Generalised Anxiety Disorder 7 Questionnaire (GAD-7), The Patient Health Questionnaire 9 (PHQ-9) and if patients reached cut-off for PTSD on the PCL-5 they were interviewed with the Clinician Administered PTSD Scale 5 (CAPS-5). Each person's total scores on each of the above questionnaires was compared in order to build a psychological symptom profile for every patient. Results: Thirty-three patients were recruited (18 males and 15 females; Age: M= 68.32). Anxiety and depressions scores were higher in the Italian (Anxiety: M= 8.73; Depression: M= 9.30) rather than in the English patients (Anxiety: M= 5.18; Depression: M=6.5). Three English and two Italian patients were interviewed further because they reached the PCL-5 cut-off score for probable PTSD and 17 registered scores of at least 10. In particular, 47.05% and 70.58% of the Italian patients reported intrusive memories of the stressful experience and strong negative feelings, while loss of interest in activities and feeling upset when remembering the moment of the diagnosis were symptoms present in the 75% of the English subjects. Conclusions: Sub-syndromal PTSD symptoms had a similar prevalence to anxiety and depression in patients with a recent diagnosis of MND. Further studies are needed to explore factors that contribute to these symptoms as well as the way of best delivering the MND diagnosis.

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D05 The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) in Alzheimer's Disease and behavioural variant Frontotemportal Dementia

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Although the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was developed to assess the cognitive changes typically affected by the frontotemporal syndrome observed in ALS patients, its sensitivity and specificity to diagnose dementia in populations without ALS has not been reported. In this study we assess how successful the ECAS is at differentiating between patient populations with Alzheimer's Disease (memory variant) and Frontotemporal Dementia (behavioural variant) to a healthy control group. Objective: Measure the sensitivity and specificity of the ECAS to different diagnoses of dementia Methods: 30 patients diagnosed with probable Alzheimer's Disease (AD), 15 patients diagnosed with the behavioural variant of frontotemporal dementia (bvFTD), and 40 healthy participants matched in age and education undertook the ECAS. Results: In the analysis of the ECAS cognitive data, AD and bvFTD patients scored significantly different than the control group in the total score of the ECAS, and in the domains of Language, Fluency, Executive and Memory. But only AD patients were impaired on the Visuospatial domain. In the AD group the cut-off of 105 in the ECAS total score had a sensitivity of .975 and a specificity of .967 .The cut-off of 24 for the Non-Specific score had a sensitivity of .950 and a specificity of .967 .In the bvFTD group the cut-off of 105 in the ECAS total score had a sensitivity of .975 and a specificity of .933 ,while a cut-off of 77 for the ALS-Specific score had a sensitivity of .975 and a specificity of .933 . In the analysis of the behavioural data, most AD patients have two behavioural changes, whereas bvFTD patients tend to have five behavioural changes. Conclusions: The ECAS shows good sensitivity and specificity as a diagnosis tool for Alzheimerâ€™s Disease and for the behavioural variant of frontotemporal dementia. The inclusion of a behavioural interview in the ECAS makes it particularly suitable to screen frontal lobe disorders.

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D06 The relationship between cognitive impairment and motor phenotypes in ALS: A population-based study

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Background. Amyotrophic Lateral Sclerosis (ALS) may present with different motor phenotypes. It is associated with cognitive impairment in half of cases, ranging from frank Frontotemporal Dementia (FTD) to mild deficits. We aimed at evaluating if motor phenotypes differ according to the frequency and severity of cognitive deficits. Methods. 1,173 incident ALS cases from the Piemonte and Valle d'Aosta Register for ALS were eligible from 2009 to 2016. 63% of patients (N=751) underwent neuropsychological assessment and were enrolled. According to Strong et al (2009), patients were classified as ALS-FTD (19.5%), ALS with cognitive impairment (23.7%), ALS with behavioural impairment (4.5%), ALS with normal cognition (52.3%). Motor phenotype was classified as lower motor neuron prevalent (22.2%), upper motor neuron prevalent (14.7%), classic (33.4%), bulbar (29.7%). The association between cognitive impairment and motor phenotype was assessed by using stepwise backward logistic regression analysis, adjusted for sex, age at onset, education, hypertension, diabetes mellitus, marital status, and C9orf72 expansion. Results. 54.6% of patients were male, with an average age at diagnosis of 67.0 (SD 10.3). Sixty-one patients carried the C9orf72 expansion (8.8%). Significant associations were detected only for bulbar patients, for whom the likelihood of developing any grade of cognitive impairment was 88% higher than that of non-bulbar patients (OR=1.88; 95% CI=1.30-2.70), while the risk of developing FTD was two-fold than that of non-bulbar patients (OR=2.17; 95% CI=1.40-3.37). Conclusions. Bulbar patients showed a higher risk of developing cognitive impairment, especially FTD, compared to non-bulbar patients, that seemed relatively less vulnerable to cognitive decline.

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D07 Behavioural correlates of attentional function in ALS patients

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Background: Past studies demonstrated a significant overlap between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) and report cognitive impairment in up to 30% of ALS patients (Rippon et al. 2006). Although executive impairment in ALS have been shown in several studies (Beeldman et al. 2016), impairments of attentional control have not been extensively examined. The aim of this work was to test if ALS patients without clinical evidence of cognitive dysfunction would show behavioral attentional deficits performing a modified version of the Attention Network Test (ANT) (Fan et al. 2005). Methods: A cohort of 19 ALS patients and 18 matched controls took part in the behavioral study and also underwent a neuropsychological screening using ECAS (Abrahams et al. 2014). In a modified version of the ANT (adapted from Firbank et al. 2016), our study participants were instructed to press a button in the direction of the majority of four arrowheads. There were two different conditions (congruent, incongruent) and the latter consisted of two levels of difficulty. Statistical analysis included a comparison of absolute reaction times as well as of reaction time differences such as executive effect (incongruent - congruent), conflict effect (incongruent hard - incongruent easy) and alerting effect (no cue - cue), and of ECAS performance. Results: Regarding absolute reaction times, ALS patients show a consistently longer reaction time compared to controls. However, there was no difference in relative reaction time differences, such as the executive effect, conflict effect or alerting effect (all p-values > 0.05). Likewise, in our cohort the results of the ECAS were comparable. Discussion: In order to eliminate the bias of motor impairment within the ALS group we did not take the absolute reaction times into account but focused on relative reaction times. In both groups we found intact alerting, executive and conflict effects, as indicated by a modulation of the reaction times by task condition. However, we did not find significant differences of attentional-executive performance between patients and controls. This may either reflect the absence of a difference between the two groups regarding attentional functions or may be due to methodological limitations such as sensitivity of the task and selection bias. Our future analysis will concentrate on association with clinical variables and brain activations to detect subclinical abnormalities.

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D08 The rate of weight loss at diagnosis in ALS is more important than BMI in predicting outcome

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Objective: Body Mass Index (BMI) decline is associated to ALS, but previous studies have given contradictory results about its role on the disease course. The aim of this study is to assess the relative role of BMI and rate of weight loss as prognostic factors in ALS. METHODS: A total of 650 patients diagnosed with ALS during the period 2007 - 2011 from the Piemonte/Valle d'Aosta Register for ALS were included. ALS diagnosis was based on the revised El Escorial diagnostic criteria. Disease severity was assessed with the ALSFRS-R scale. BMI levels were collected at the time of diagnosis and categorized according to the WHO classification. RESULTS: BMI at disease onset resulted to be an independent prognostic factor for underweight patients (BMI <18.5, p35.0, p= < 0.05). Lower premorbid BMI among underweight patients and higher pre-morbid BMI in obese (Class II-III) patients predicted a shorter survival. Patients' outcome was significantly associated to the rate of weight loss at diagnosis. Higher mean monthly BMI loss before diagnosis predicts a shorter survival ($p < 0.005$) and a faster disease progression calculated by the decline in ALSFRS-R score ($p < 0.005$). The rate of BMI loss predicts a faster progression of motor symptoms ($p < 0.005$) but did not correlate with the severity of bulbar symptoms. These findings remained significant after a multiple regression analysis was performed to adjust for age, gender, history of smoking, BMI at diagnosis, FVC at diagnosis and type of onset (bulbar or spinal). A subgroup of patients with spinal onset without bulbar signs at time of diagnosis had a BMI loss comparable to that of bulbar onset patients, indicating that in these patients a different mechanism other than malnourishment was the cause of weight loss. DISCUSSION: We tested the hypothesis that pre-morbid BMI and change in BMI before diagnosis influence ALS course on a large population-based cohort of ALS patients. Pre-diagnostic BMI had a non-linear association with survival, with BMI levels 35.0 was associated with a shorter survival. A decrease in BMI was associated with a faster clinical course of ALS: higher weight loss before diagnosis strongly correlated with a faster functional decline and a worse prognosis. The evaluation of BMI loss at diagnosis may help predict disease progression in ALS patients and may identify a group of patients more likely to have a more aggressive disease.

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D09 Percutaneous endoscopic gastrostomy with noninvasive mechanical ventilation in patients with amyotrophic lateral sclerosis

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Introduction: Patients with Amyotrophic Lateral Sclerosis (ALS) eventually require enteral nutrition trough a gastrostomy tube. This surgical procedure may imply an important perioperative respiratory risk of complications. Aim: To describe the results of the percutaneous endoscopic gastrostomy (PEG) tube placement with the use of noninvasive positive pressure ventilation (NPPV), in a series of patients with ALS. Methods: Longitudinal, observational and retrospective study of a series of ALS patients with a PEG performed in the past 10 years (2007 – 2017) in a third level hospital, reference center for ALS. Epidemiologic, anthropometric, respiratory, neurologic and nutritional data previous to the procedure were collected, as well as early (30 days) complications regarding the surgical procedure. Before the PEG a pre-anaesthetic evaluation was performed and optimizations of the secretions with coughassist devices as well as adaptation to NPPV were also achieved. The PEG was performed in operating rooms with deep sedation and continuus cardiorespiratory monitorization. A special facial mask designed for endoscopic techniques was adapted to the NPPV device. After the procedure, patients had a brief stay in a recovery room, and then they were transferred to the hospitalization room. Results: The procedure was performed in 59 patients, 24 (40%) of them male, with a mean age of 60 (±13) years. Affectation was mainly bulbar in 52% of the cases (n=31). Before the intervention the following data was registered: BMI 20 (19-23) kg/m2, ALSRFS-R 25,5 (17-32), FVC 67% (47% – 80%) and supine postion drop of 22% (10% - 39%) FVC in the spirometry. The time between the diagnosis and the procedure was 14.6 months (9.5-23). Six early complications were observed: pneumoperitoneum (n=2), peritube bleeding (n=2), mild tearing of the cardia (n=1) and upper digestive tract bleeding (n=1), all of these were self-limited in the following 48 hours. One patient presented with an infection as a late complication, with a good response to antibiotic treatment and tube change. There were no complications related with the ventilatory support and there were no deaths regarding the procedure. Survival after 1 year of the technique was 9,7 months (IC 95% 8,7-10,8). Conclusions: In this series of ALS patients, the PEG tube placement with NPPV support in ALS patients is a safe procedure, even in severe neurologic and respiratory affectation.

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D10 Organ donation after cardiac death in ALS patients: Protocol and experience in a tertiary center in Spain

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Introduction The survival in amyotrophic lateral sclerosis (ALS) largely relies on the ventilation support treatment and its withdrawal at the patient's request is a right. These patients are candidates for donation in controlled asystole and previous reports have shown that this process is feasible and ethically acceptable. We aim to present a protocol for organ donation after cardiac arrest in ALS patients and the preliminary experience in a tertiary center in Spain. Methods Health care professionals (neurologist, psychologist, pulmonologist homecare physician) of the Hospital la Fe ALS Unit, developed a protocol for the end of life care of ALS patients, which was approved by the Bioethics Committee of our Institution. As a part of the advanced directives, patients were informed about the possibility of donating organs and their wills were recorded. Since April 2016, this protocol has been implemented in collaboration with the Hospital at Home Unit and the Intensive Care Unit. Results Between April 2016 and December 2017, 45 patients completed the advanced directives document. Twenty-five (55%) of them expressed their willingness to donate organs for transplantation if possible, and the desire to help other patients was the reason given by a great majority of them. Fifteen of these 25 ALS patients have died up to now. In eight of them the organ donation was not accomplished because patients did not meet the required medical criteria. In the other seven patients, the organ donation protocol was initiated when they requested sedation and ventilator support withdrawal. The reason for their request was the worsening of dyspnea in six non-invasive ventilated patients who did not want to progress to tracheostomy; and the loss of life meaning in one invasive ventilated patient. The explant after cardiac arrest could be successfully conducted in 6 patients. Their relatives could accompany them during the whole process and expressed their satisfaction. Conclusions The organ donation after cardiac arrest is ethically desirable and technically feasible in ALS patients requesting a withdrawal of ventilatory support, although each case must be assessed individually. Most patients wish to be donors and, in those cases in which the donation was carried out, the family was satisfied with the process.

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D11 Estimating the overall prevalence of ALS and of different stages of cognitive impairment in Catalonia. A retrospective population cohort design

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Background: The prevalence of ALS has been estimated to be between 5 and 5.4 per 100,000 inhabitants. However, there is great geographical heterogeneity. There are not estimates of the prevalence of patients with ALS according to various stages of cognitive impairment. Our objectives are to estimate the overall prevalence of ALS and estimate this prevalence for various stages of cognitive impairment. Methods: In our study, we use a population-based retrospective cohort composed of 391 ALS patients, diagnosed between 2011 and 2016. For the estimation of prevalence we use a two-part model. In the first stage, using a mixed logistic regression, we estimate the probability that a subject appears in the sample. In the second, we use these probabilities as weights in a mixed generalized linear model of binomial response, to estimate the prevalence. Both parts are estimated simultaneously following a Bayesian perspective, using the INLA approach. In the model we control for observed (structure of age and sex of the population, distance to the reference hospital, among others) and unobserved confounding (random effects capturing individual heterogeneity, spatial and temporal dependence). The stages of cognitive impairment (CI) considered are: no cognitive impairment, mild behavioural CI, mild cognitive CI, mixed mild CI, frontotemporal dementia and Alzheimer's type dementia Results: As preliminary results, 55.5% are men and 44.5% are women. The age at onset of symptoms is 65.7 years ± 12.35 (median 67 years, IQR:58-75). Regarding the phenotype, spinal onset is observed in 50.4%, bulbar in 18.9% and respiratory in 3% of the patients. 37.1% of patients have no cognitive impairment, 32.9% mild cognitive CI, 15% mixed mild CI, 7.9% frontotemporal dementia, 5% mild behavioural CI and 1.4% Alzheimer's type dementia. We estimate the crude overall prevalence in 4.35 per 100,000 inhabitants (95%CI 2.74-5.96 per 100,000 inhabitants). The crude prevalence for cognitive impairment stages are: 1.00 (95%CI:0-2.13) mild cognitive CI; 0.73 (95%CI:0-1.38) no cognitive impairment; 0.23 (95%CI:0-0.49) mixed mild CI; 0.11 (95%CI:0.02-0.20) frontotemporal dementia; 0.03 (95%CI:0-0.08) mild behavioural CI; and 0.03 (95%CI:0-0.07) Alzheimer's type dementia (all per 100,000 inhabitants). Conclusion: Our preliminary results indicate that the prevalence of overall ALS will not be very far from that reported by the literature.

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D12 The MotOrtose project – Development of a motorized upper extremity orthosis for ALS

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Motor neuron disorders such as amyotrophic lateral sclerosis (ALS) are neurodegenerative disorders with a clinical hallmark of progressive impairment of motor functions. In several patients, loss of arm function occurs early in the disease course. The «flail arm syndrome» is a distinctive variant of motor neurone disease (MND), affecting about 10 % of patients with a higher proportion of men than in the general MND population. Regardless of MND subtype, early loss of arm function constitutes a major challenge to the maintenance of activities of daily living as well as quality of life. Care includes a multidisciplinary team which provides technical aids tailored to the loss of function experienced by the patient. Whereas loss of lower extremity function is substituted with electric wheelchairs securing mobilization, the availability of aids to substitute upper extremity function is limited. The brother of a patient with spinal onset ALS is professor emeritus of cybernetics. In collaboration between the Department of Mechanical and Industrial Engineering, Norwegian University of Science and Technology, and the Department of neurology at St Olav's University Hospital, a prototype of a motorized combined elbow and shoulder orthosis has been developed. At project initiation, the project goal was to make a motorized exoskeleton with a range of movement sufficient to move a paralysed arm from a vertical downward position up to the face. The total weight should be reduced to an absolute minimum. A prototype was finished meeting the project goals. Movement is secured by two actuators localized on the upper arm orthosis. Sufficient weight distribution was obtained by means of a carbon fibre torso exoskeleton. The control mechanism was originally manual, but due to paresis of the other arm, a pedal with two rods is now in use. At present, we are developing voice control as an alternative mode of actuator engagement. We are in the planning process of developing a second orthosis to a patient with flail arm syndrome, and in the process stream line the production in collaboration with the local technical aids centre and actuator component manufacturers. We will provide pictures and a video demonstration of the prototype orthosis.

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D13 The development of a Norwegian ALS registry

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Several European ALS registries have included patients on a national or regional scale, some for decades, providing important epidemiological and clinical information for quality of care assessments and research purposes. The Nordic countries are arguably particularly well suited for registries with their transparent structure and publicly financed health systems. In 2015, a research group at Karolinska hospital in Stockholm developed a Swedish clinical ALS registry. At the university hospital in Trondheim, Norway, we have developed a similar tool in 2017, ready to be launched from the spring of 2018 at a regional level, with an aim of national coverage by the end of 2018 if governmental approval and funding can be obtained. Patient consent is mandatory on enrolment. The variable list is selected to facilitate comparison with the Swedish registry. The core data set includes data on neurologic progression, treatments, ALSFRS-R scores, HADs scores and critical information such as attitude towards invasive ventilation. Some variables such as HADs, weight, disease complications, follow-up in primary care and use of alternative medication not provided by the health services can be collected by patents or relatives as Patient Related Outcome Measures - PROMS. The technical solution provides longitudinal presentations of core variables for the individual patient, thus giving the clinician a simple overview of the status of each patient. This might structure follow up, but also ease recruitment to clinical trials. In addition, information is uploaded to an aggregated group level for quality of care assessment purposes on the regional and national level. The main quality indicators for these assessments will be equal access to multidisciplinary teams and supportive treatment. In particular, the use of gastrostomy and non-invasive ventilation , can be evaluated according to national and international best practice guidelines. The presentation will include illustration of the user interface.

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D14 Do ALS motor phenotypes develop stochastically?

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Background and aims: Amyotrophic Lateral Sclerosis manifests with various motor phenotypes. Our aim was to assess whether these phenotypes are determined by detectable factors. Methods: ALS incident patients (N=2,702) included in the PARALS (an Italian regional registry of ALS) from 1995 to 2014 were enrolled. Six motor phenotypes were considered: classic, prevalent upper motor neuron, flail arm, flail leg, respiratory, classic bulbar, prevalent upper motor neuron bulbar (Chiò, JNNP 2011). Logistic regression analysis was performed, adjusting for gender and age (ten-year age classes). The outcome was represented by dummy variables: spinal vs bulbar phenotypes as macro-categories; each spinal phenotype (classic, flail arm, flail leg, prevalent upper motor neuron, respiratory) vs bulbar phenotypes; each bulbar phenotype (classic and prevalent upper motor neuron) vs spinal phenotypes. Results: Males showed a probability of developing a spinal form 72% higher than females (OR=1.72; p=0.000). Among patients over 60 years, the spinal onset was less frequent than the bulbar one (test for trend in subsequent ten-year age classes: p<0.0001). This finding was particularly strong in females, with ORs between 5.40 (60-69 years) and 9.10 (over 80 years). Respiratory and flail arm phenotypes were more common in males, with a probability more than ten-fold and more than two-fold than females respectively (OR=11.72 and OR=3.39). The likelihood of the pyramidal bulbar phenotype resulted more than two-fold in females compared to males (OR=2.20; p=0.0001), without differences among age classes. Conclusion: ALS motor phenotypes seem to arise from a combination of patients' gender and age.

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D15 A pilot study of voice banking in amyotrophic lateral sclerosis patients

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One of clinical consequences of the bulbar dysfunction in Amyotrophic Lateral Sclerosis (ALS) is the progressive loss of ability to speak and of effective communication with devastating social and psychological effects. The strategies based on Alternative Augmentative Communication (AAC) allow patients to maintain an useful communication using a synthetic voice (ST) with a direct improvement of the Quality of Life (QoL). However, the ST produced by the AAC is often perceived as impersonal and not representative of the person's identity. In the recent years, a new strategy defined voice banking (VB) has been defined to overcome this problem. VB is based on the recording of a list of phrases with voice of patients, when speech is preserved. This recording is converted to create a personal ST to be used with the AAC devices. The primary aim of the study was to determine the feasibility and psychological impact of VB in a group of ALS patients. We recruited ALS patients satisfying the following criteria: a diagnosis of definite o probable ALS according El Escorial Criteria, a normal cognitive status evaluated by Edinburg Cognitive Assessment ALS Screen (ECAS) and a preserved speech as represented by an ALSFRS-R language subscore ≥3 points and a ECAS score in the language task > 15.56. Before and after the process of recording each patient underwent a battery of tests to assess QoL and the psychological and cognitive status; they also received the material and instructions to access VB at home. For each recording, we considered the number of sentences and the time spent. VB was proposed to six patients, one dropped-out and three completed the registrations in different time intervals. Among these three, two showed mild levels in depression and anxiety, while one showed a stable mood. The fourth subject showed mild levels in depression and anxiety and he felt the VB experience as a constant reminder of the disease progression, thus he didn't complete the registration. The fifth subject stopped the VB due to a significative and rapid worsening of the speech impairment. In conclusion, this pilot experience, even if based on a small group of patients, underlined some important findings that may influence a VB strategy in ALS patients, such as the timing of the proposal, the instruction for recording and the psychological impact. Our next step is to conduct a second study with a bigger number of patients to better explore the VB in ALS.

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D16 Design and implementation of an augmented reality device for environment control in amyotrophic lateral sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a group of rare neurological diseases that mainly involve the nerve cells (neurons) responsible for controlling voluntary muscle movement. The disease is progressive, meaning the symptoms get worse over time. Currently, there is no cure for ALS and no effective treatment to halt, or reverse, the progression of the disease. The success of the rehabilitation approach depends on the active participation of the patient who should be a full partner in the rehabilitation process even during the advanced stages of the disease, in which the majority of patients can reach a 'locked-in' state. The project aim was to develop a new device called ECO ALS system, based on a new eye-tracking system integrated on smartglasses for an augmented reality called EyeSpeak, that gives to the ALS patient the possibility to control the environment and to manage in autonomy a power wheelchair and an electric bed. We evaluated the feasibility and usability of the device in ALS patients in advanced stages of disease. The information collected during the design and development of device, following the basic principles of the human-centered design, were useful to better understand the functional advantage of this new class of assistive technologies for patients with high level of disability. The project included a closed collaboration between a tertiary multidisciplinary highly specialized ALS Center and an assistive technology company committed to the development of software and new technologies for people with severe motor disabilities. The study included a complete visual analysis of the ALS patients to well define and characterized possible interfering factors related to the visual system. The study included ALS patients in advanced stage of disease for the implementation and development of the device. The next phase will include ten patients that will test the final version of the device. Patients and their caregivers will be evaluated to assess the level of satisfaction related to this new assistive Technology, its psychosocial impact, and the changes of quality of life and caregiver's burden related to the use of the device. We expect to create a device that increases autonomy and that can improve people's and caregivers' quality of life.

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D17 The effects of intensity, duration and time-since-quitting on the association between total cigarette smoking and ALS risk: Euro-MOTOR

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Objective – We investigated the association between smoking and the risk of amyotrophic lateral sclerosis (ALS) in a pooled analysis of three population-based studies and explored possible effect modification by smoking intensity, duration and time-since-quitting. Methods – ALS cases and matched controls were recruited in the Netherlands, Italy and Ireland (Euro-MOTOR project, 2010-2015). Demographics, detailed lifetime smoking histories and information on other lifestyle factors were collected via paper questionnaires. Logistic regression models were applied for smoking status, intensity (cigarettes/day), duration (years), cigarette pack-years and time-since-quitting (years), adjusted for age, sex, alcohol and education. We further applied flexible excess odds ratio (OR) models, which was linear for pack-years, but potentially non-linear for intensity, duration and time-since-quitting. Results – Analyses were performed on 1,410 cases and 2,616 controls. Pack-years were positively associated with ALS risk, with an OR of 1.26 (95% confidence interval (CI) 1.03-1.54) for the highest quartile compared with never smokers. This association appeared to be predominantly driven by smoking duration: the model for duration showed a clear positive trend with ALS (p=0.001) while smoking intensity did not (p=0.862). Time-since-quitting was inversely related with ALS (p<.0001). The excess OR per cigarette pack-year decreased with time-since-quitting smoking, until about 10 years prior to the disease onset. Interpretation – Our findings provide further support for the causal association between smoking and ALS. Cigarette pack-years alone may not be sufficient to capture the effect of different smoking patterns on the association with ALS. Particularly time-since-quitting appears to be important to take into account.

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D18 Factors influencing diagnosis delay in ALS patients referred to a secondary center for neuromuscular diseases in Poland.

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Background: Due to a lack of reliable disease biomarkers, an early diagnosis of ALS still raises difficulties. The diagnosis delay results from an inter-play between patient-linked symptom recognition and the referral pathway. The formal-diagnosis is often delayed not only due to uncertainty but also a redundancy to break-through the unfavorable news. OBJECTIVES: The aim of this study was to analyze factors influencing formal-diagnosis delay in the Polish population. PATIENTS and METHODS: We retrospectively analyzed data of 541 consecutive ALS patients fulfilling the El Escorial criteria referred to the Neuromuscular out-patient clinic between 07/2003 and 06/2017. The mean age at symptoms onset was 55.6±12.7 years, the disease duration at the first visit – 22.8± 27.1 months, and the M:F ratio 1.1:1. The diagnosis delay was defined as time from symptoms onset to the final formal diagnosis of amyotrophic lateral sclerosis (G12.2), which permitted a state-refund of riluzole treatment. RESULTS: The mean formal-diagnosis delay was 13.1±10.3 months. It was shorter in bulbar (11.4±23.2 months) compared to limb onset ALS (13.6±10.3 months), in sporadic compared to familial cases (13.1±10.2 vs 14.7±9.5 months) and in patients living in cities as compared to villages (12.5±10.3 vs 15.2±10.3 months, respectively). It was also slightly shorter in female (12.6±10.2 months) compared to male patients (13.5±10.3) and in patients aged 40 years (13.2±10.3 months). There was no impact of the patients' education and martial status. CONCLUSIONS: ALS patients experience significant delay to obtain the final formal diagnosis of ALS. Longer diagnosis delay is associated with limb onset, FALS, male gender, age > 40 years and village provenience. There is a high need to increase the physicians' consciousness with respect to the need to give a formal ALS diagnosis as soon as the patients fulfill the diagnostic criteria.

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D19 Care Audit Research and Evaluation for MND (CARE-MND): An electronic platform for motor neurone disease in Scotland

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Background: Motor neurone disease (MND) has a significant impact on patient disability and healthcare services. Recent NICE Guidelines highlight imperative management goals (1). Since 1989, the Scottish MND Register (SMNDR) has provided a resource for prospective clinical data collection. However, data capture was reliant on research personnel and was difficult to maintain. We present a completed audit cycle of data capture including the implementation of an alternative platform. Aims: 1) to evaluate SMNDR data capture 2011-14; 2) to develop and implement a user-friendly national platform, capturing routinely-collected clinical data; 3) to align with NICE Guidelines; 4) to sustain CARE-MND for improved care and research. Methods: Seventeen data fields were audited in cycle one. Following identification of suboptimal capture, a CARE-MND proforma and electronic platform was introduced. CARE-MND development coincided with NHS Scotland and Scottish Government-funded doubling of the number of MND care specialists. Data fields were aligned to NICE recommendations, and re-audited for data-input 2015-17. Pre- and post-intervention data capture were compared using Z-test of proportions. Results: SMNDR data capture ranged from 4-95%; average 49%. CARE-MND capture ranged from 32-98%; average 78%. 15/17 fields were significantly more complete post-intervention ($p<4.19x10-5$). "Place of death" capture remained high (95%, 97%). "Forced Vital Capacity (FVC)" capture remained low (34%, 32%), likely because of recent replacement by transcutaneous CO2 monitoring. Conclusions: As a result of this audit, all MND care specialists have incorporated CARE-MND into clinical practice. Ongoing data entry is audited monthly. Through CARE-MND, national audits of cognition, gastrostomy, respiratory interventions and riluzole are ongoing, with the aim of developing care protocols for harmonised service provision. Stratification of the MND population is facilitating research, including clinical trials. We have established a platform which integrates care and research and which might have utility for other neurodegenerative diseases. 1. National Institute for Health and Care Guidance. Motor neurone disease: assessment and management | Guidance and guidelines | NICE [Internet]. NICE guideline. NICE; 2016

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D20 Religiosity in Polish and German patients with amyotrophic lateral sclerosis

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Introduction: Due to inevitable disease progression and shortening the life-span, ALS patients require broad psychosocial support. Religion and faith are significant sources of support for the ill. The aim of the study was to analyze religiosity and its correlation with socio-demographic, clinical and psychological factors in Polish and German patients with ALS and country-specific controls. Material and Methods: The total of 265 ALS patients (n=122 Polish and n=143 German) and 200 age-, gender- and education level-matched healthy controls (n=100 each) were included into the cross-sectional study. The disease duration ranged from 3-88 months. The Polish and German ALS patients were matched for age, gender and functional impairment (mean 59.4/62.8 years; 54/53% males, mean ALSFRS-R 36/36, respectively). Public, private and overall religiosity was assessed by the Idler's religiosity scale (IIR). The quality of life (QoL) was measured with the anamnestic comparative self-assessment (ACSA) and the Schedule for the Evaluation of the Subjective Quality of Life (SEIQoL), depression by the ALS-Depression-Inventory 12 Item. Results: Only 5% of Polish and 19% of German patients declared themselves atheists. In both Polish and German samples the public, private and total religiousness were similar among patients and controls (medians: 6/6/12 vs 6/5/11 for Polish and 5/5/10 vs 5/5/10 for German, respectively). Polish ALS patients had significantly higher scores on all measures of religiosity compared to German patients. The patients' religiousness didn't correlate with physical impairment (ALSFRS-R), disease duration and pain measures, while the private and total religiosity positively correlated with age ($p<0.05$). There was a negative correlation between education and religiosity in Polish, but not in German ALS patients. The Polish female patients showed higher level of public, private, and total religiosity as compared to male patients (median: female 6/6/13 vs male 5/5/10). We found no correlation between depression and any aspect of religiousness, and only a weak positive correlation between public, private, and total religiousness and subjective QoL in Polish, but not in German patients. Conclusions: We found no correlation between religiosity and studied clinical and psychological aspects of ALS. Religiousness was however significantly linked to the country of origin, age and education suggesting the influence of cultural and social conditions.

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D21 Modelling individual amyotrophic lateral sclerosis disease courses in different centers using the D50 progression model

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Introduction: Progression in ALS varies between and within individuals across time; the D50 model addresses this phenotypic complexity and reduces noise associated with current clinical indices. It uses functional profiles to describe progression independent of assessment time. Objectives: The model was developed using a cohort of 400 patients tracked in one center and validated using the PROACT database. We now aim to extend validation and develop new indices by applying the model to data from 6 geographically distinct centers. Methods: We used iterative least-square fitting of all available ALSFRS-R scores to describe disease progression using a sigmoidal state transition from full health to complete functional loss. The model yields 3 summative descriptors of disease aggressiveness: D50 (time taken for ALSFRS-R score to reach 24), dx (time constant of ALSFRS-R decay), and relative D50 (calculated value describing individual disease covered in reference to D50, 0 = disease onset and 0.5 = time-point of halved functionality). The model provides 2 local descriptors of disease activity: calculated functional loss (cFL) & functional state (cFS). Results: Summative disease descriptors were calculated for all 4838 patients in the PROACT database; D50 was significantly correlated both to survival (r = 0.654) and first (r = 0.601) and last (r = 0.772) recorded PRs. Next, the model was applied to 4091 patients from 6 centers; summative and local disease descriptors were calculated for all patients. rD50 aligns patient cohorts from centres in terms of elapsed disease course, thus allowing comparability despite different time scales. Patients were stratified into mathematically derived phases I & II (early semi-stable & early progressive phases) and Phases III & IV (late progressive & late semi-stable phases). Centers had sampled patients mostly in Phases I and II. D50 and dx were linearly correlated in all centers (r: 0.93–0.99) with very similar steepness (a: 0.40–0.50) and offset (b: 0.80–1.54), confirming the model validity. Conclusion: The D50 model provides meaningful descriptors of overall disease aggressiveness, local disease activity, and a unified linear scale to describe disease progression. It a) offers alternative reference points to survival, b) allows the staging of individual events, c) provides a way to pseudo-longitudinally interpret cross-sectional data and d) efficiently compares the composition of cohorts from different geographic regions.

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D22 Patterns of spreading of weakness in amyotrophic lateral sclerosis based on patients' reports

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Introduction In amyotrophic lateral sclerosis (ALS), symptoms start focally in a body region and spread progressively to other segments. We analyzed the pattern of regional spreading of weakness according to patients' histories. Patients and methods We analyzed the histories of 250 patients diagnosed with ALS in our ALS Center between 2007 and 2018. Only patients reporting a focal onset of weakness and a subsequent involved region were included. The following regions were considered: bulbar, upper limbs, and lower limbs. Results Site of onset was bulbar (B) in 40 and spinal in 210 patients (upper limbs (UL), n = 99; lower limbs (LL), n = 111). Four different spreading patterns were recognized: contiguous descending (from B to UL, n = 27; from UL to ipsilateral LL, n = 10), contiguous ascending (from UL to B, n = 12; from LL to ipsilateral UL, n = 27), contiguous horizontal (from UL to contralateral UL, n = 73; from LL to contralateral LL, n = 72), and non-contiguous (from B to LL, n = 13; from UL to contralateral LL, n = 4; from LL to contralateral UL, n = 4; from LL to B, n = 8). Overall, contiguous patterns overwhelmingly predominated over non-contigous ones (221 vs. 29). For both UL- and LL-onset, the horizontal pattern similarly predominated over the contiguous vertical (ascending or descending) one (UL: horizontal, n = 73; contiguous vertical, n = 22; LL: horizontal, n = 72; contiguous ascending, n = 27). For upper-limb onset, contiguous ascending and descending patterns were similarly represented (n = 12 and n = 10, respectively). Discussion In most ALS cases the spreading of symptoms is contiguous, which reflects contiguous spreading of pathology. This would be consistent both with a cortical and with a spinal spreading process. The same is true for the predominant horizontal pattern observed in limb-onset cases. When considering an intraspinal spreading mechanism, this horizontal predominance would reflect the shorter distance which a putative pathogenic agent would have to cover to reach the corresponding contralateral spinal motor neurons than to reach another ipsilateral cord segment. In case of an intracortical spreading mechanism, the predominant horizontal pattern would point to a critical involvement of callosal interhemispheric connections. Importantly, our analysis has the main limitation of being based exclusively on patients' reports, which does not necessarily reflect the exact sequence of spreading of pathology.

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D23 Characterising the metabolic profile of ALS: Results from the EuroMotor study cohort

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Amyotrophic Lateral Sclerosis (ALS) a devastating disease, with incidence ranging from 2 to 3 cases per 100,000 individuals in Europe. Currently, there is no cure for ALS and a lack of validated targets reflects our inadequate understanding of disease mechanism and progression. The aim of the FP7 Euro-MOTOR study is to identify novel causes of ALS using a comprehensive systems biology approach. Within this study a large-scale, pan-European population-based metabolomic study has been conducted. The Euromotor metabolomics cohort consists of 1649 samples from the Netherlands, Italy and Ireland. Upon final recruitment to the study and sample collection it was possible to fully match 726 case-contol pairs by centre, age (Â± 5 years) and gender, with data generated for a further 159 cases unmatched. This provides the maximum potential to develop prognostic markers since all ALS cases were available were analysed. We have carried out metabolomic analysis of serum samples using the targeted AbsoluteIDQTM p180 platform (Biocrates Life Scieneces AG). The AbsoluteIDQTM p180 kit allows the targeted analysis of amino acids, biogenic amines, acylcarnitines, sphingolipids and glycerophospholipids. The presence of ALS has a clear impact on the serum metabolome, across three different European populations Netherlands, Ireland, and Italy. Major differences were identified in the overall profile of amino acids, carnitines, and creatinine likely reflecting changes in muscle metabolism and perturbation of Î²-oxidation activity in patients. The results also suggest differences also to the lipid profile with emphasis to di-acyl-phosphocholines with PUFA lipids, consistent with perturbation of lipid metabolism or nutritional contributions to lipid composition. Moreover, OPLS-DA model was constructed from a discovery cohort that could successfully predict the ALS control status in the replication set. The classification performance in the replication set translates to this metabolomic blood test having a likelihood ratio of ~4, i.e. a positive test result from the model makes it 4 times more likely that a patient has ALS. Based on the identified metabolomic signature of ALS, survival models were constructed based on metabolic biomarkers. Predictive multivariate metabolic models were constructed in order to predict disease stage, outcome, and also disease progression based on functional ALSFRS-R scores.

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D24 NeuroGUIDization of PALS population for patient-centric research and care

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Objective: Establish an approach, technological platform and processes to facilitate international collaboration in patient-centric research and care while protecting patients' privacy BACKGROUND: With numerous clinical research studies conducted in ALS/MND, there is an increasing need to link information from multiple sources on the same patients. To reach this objective, the Neurological Global Unique Identifier (NeuroGUID™) generation platform for international research community was deployed. NeuroGUIDs allow merging data acquired on research participants in multiple studies and linking to other informational sources. While thousands NeuroGUIDs are currently in use, they should never be used in databases that also store PII/PHI, like EHR systems, patient registries, or mobile apps, as this may re-identify patients. DESIGN/METHODS: The NeuroGUID technology and platform, allows to link separate datasets into coherent harmonized data-sharing environment, while maintaining compliance. A domain-specific central authority for generating NeuroGUIDs suitable for inclusion in de-identified datasets is set up by Neurological Clinical Research Institute. A System-specific Transactional Anonymous PIN (NeuroSTAmP™) is a unique research participant identifier per application (e.g., EHR) per institution (e.g., MGH). NeuroSTAmPs could be created by using the same information as in NeuroGUID™ generation or, if NeuroGUIDs are known, by specifying NeuroGUIDs instead. Only NeuroGUID™ server knows how to link multiple NeuroSTAmPs to a NeuroGUID™. RESULTS: NeuroGUIDs are widely accepted by ALS/MND researchers. NeuroGUIDs and their derivatives (NeuroSTAmPs) are utilized to connect clinical and research data to biospecimen collections (embedded into bar-coded labels on biofluids and postmortem tissues), images (introduced into image headers), WGS files, cell lines, EHRs and mobile apps. Thanks to NeuroSTAmPs, researchers can merge information without exposing NeuroGUIDs to people or systems that have access to PII/PHI. CONCLUSIONS: NeuroGUID technology is uniquely suitable for use with de-identified datasets. It links bio-samples and images with clinical data and electronic health records. Clinicians and researchers utilize this technology, which facilitates international scientific collaboration.

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D25 Exosomes as novel therapeutic approach for ALS

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myotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease and the superoxide dismutase gene (SOD1) is one of the major ALS-associated genes. To date, therapeutic strategies for ALS are minimally effective on patients' survival and quality of life. Stem cells represent a promising therapeutic approach in the treatment of ALS. Several observations lead to the hypothesis that stem cells exert their beneficial effect through the secretion of exosomes, extracellular vesicles from 30 to 100 nm in diameter that play a fundamental role in intercellular communication. These vesicles enhance the repair of the damaged area releasing their content and could be used as a novel cell-free therapeutic approach, avoiding all the risks associated with the use of cells. In our studies, we wanted to assess the efficacy of exosomes derived from adipose stem cells (ASC) on in vitro and in vivo ALS models. We demonstrated that ASC-exosomes have a neuroprotective effect in vitro on motoneuron-like cell line (NSC-34) naïve and transfected with different human mutant SOD1 gene (SOD1(G93A), SOD1(G37R) and SOD1(A4V)). The presence of exosomes protects cells from oxidative damage, with a significantly increase of cell viability. On this basis, we wanted to assess the potential neuroprotective role of ASC-exosomes in vivo, on SOD1(G93A) murine model, monitoring the homing of exosomes after their administration. In addition, we wanted to identify the molecules responsible for this neuroprotective function. In in vivo experiments, the intravenously injection of ASC-exosomes in SOD1(G93A) mice at clinical onset until terminal stage point out that exosomes delay symptoms progression of treated animals. This data demonstrate that ASC-exosomes have a neuroprotective effect also in in vivo model of ALS, indicating a possible new approach as therapy in this neurodegenerative disease. To monitor the tracking and the homing of ASC-exosomes after in vivo administration, we set up a new protocol to label exosomes with superparamagnetic iron oxide nanoparticles, which allow their detection with a non-invasive technique as magnetic resonance imaging. Moreover, we performed the protein content characterization of ASC-exosomes, that allow us to identify some of the molecular pathways by which exosomes explain their neuroprotective function counteracting the pathogenetic mechanisms involved in ALS. Supported by AriSLA grant FGBR_7/2016.

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D26 A new view of retinoic acid's function in the neuromuscular system and its potential as a therapeutic for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a major late onset, progressive and fatal motor neuron disease. The pathogenic mechanism of the disease has not been clearly elucidated and neither has an effective therapeutic approach been developed. The retinoic acid (RA) signalling pathway plays crucial roles in several neurophysiological functions that may be therapeutically beneficial in ALS, including control of neuronal regeneration, plasticity, protection and survival. The goals of our study were to examine the expression and distribution of the proteins associated with RA synthesis and signalling in the neuromuscular system and to evaluate its potential as a target in the treatment of neuromuscular disease. The expression and distribution of the retinoic acid-synthesizing enzyme retinaldehyde dehydrogenase 2 (RALDH2) was investigated in the neuromuscular system. In addition a downstream effector of the RA signalling system was studied; the type 1 cannabinoid receptor (CB1) an integral component of the endocannabinoid system with key functions in the modulation of neuronal plasticity and survival. The synthetic RA receptor ligand EC23 was also investigated to examine its ability to modulate the contractile response of the neuromuscular tissue to RA stimulation. Our results showed that RALDH2 was expressed and distributed in every segment of the spinal cord of the adult rat with an increased expression in lumbar segments 4 and 5 where motor neurons are most abundant. Immunolabelling of transverse sections of lumbar spinal cord suggest RALDH2 labelling in motor neurons. Immunohistochemistry also confirmed the presence of CB1 receptors at the neuromuscular junction and around the entire muscle fibre. Electrophysiological recording from nerve/muscle preparations showed that 1nM EC23 enhances muscle twitch and tetanus tension in both terms of absolute muscle tension and nerve-evoked tension when synaptic transmission is impaired. Our data suggests that endogenous RA plays a role in adult neuromotor function. Further, exogenously applied retinoids can improve muscular contraction and performance of impaired nerve-muscle signalling likely to be found in diseased neuromuscular synapses. Further investigation of the RA signalling pathways are ongoing in the neuromuscular system to understand its interaction with other signalling systems and its potential association with, or amelioration of, the pathogenesis of ALS.

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D27 Machine learning tools for improving the efficiency of drug development clinical trials in ALS

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Objective: To improve the efficiency of clinical trials in ALS. Background: Starting with an ALSFRS-R machine learning model, we developed total ALSFRS-R score, ALSFRS-R subscores, vital capacity (VC), and time-to-event models, including survival, loss of speech, use of wheelchair, gastrostomy and use of NIV (using time to 50% expected VC as a surrogate). Design/Methods: ALS models were developed using the PRO-ACT database. We initially clean the data, run a preliminary random forest model for variable reduction, then refine and improve the models using gradient boosting machines. The models are validated for internal consistency using ten-fold cross validation and generalizability using external datasets. Regression models were characterized using RMSD and bias analysis and time-to-event models were characterized using ROC curves, calibration and discrimination. Results: Drug development tools have been created, including virtual controls for situations without a placebo (e.g., in early phase trials) and where a placebo is ethically challenging (e.g., cell & gene therapies with invasive procedures) or impossible to blind. In addition, we have developed an enrichment tool to decrease the heterogeneity of trial populations, target potential responders and decrease variance in primary endpoints. Finally, a novel method of randomizing clinical trials and adjusting for covariates by using predicted outcomes has been developed. The virtual control provides an objective measure of efficacy in early trials and can inform subsequent trial design, clinical trial enrichment based on predicted progression can reveal therapeutic effects hidden in the larger population, and the use of the randomization and covariate adjustment tool can reduce sample sizes or increase the power of a study. The tools have been discussed with the US FDA. Conclusions: The virtual control, enrichment, and randomization/covariate tools provide an objective measure of efficacy in early clinical trials, improve trial homogeneity, and lower sample size/increase power for drug development trials in ALS. These applications represent a significant paradigm shift with broad implications for the conduct of trials in ALS in particular and can be extended to a range of neurodegenerative diseases.

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D28 People living with ALS and their caregivers' input into drug development in Europe

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Background: There is a growing call for rigorous patient input into key areas of drug development, regulatory consideration, such as clinically meaningful outcomes and benefit/risk calculations, as well as an emerging focus on patient engagement in determinations of value for health system utilization and payment. These developments offer a critical opportunity to use established methods to ensure that patient input is appropriately integrated into drug development. Through a survey of ALS patients and caregivers in Europe, this initiative aims to gather perspectives of the burden of disease of amyotrophic lateral sclerosis (ALS) with emphasis on the loss of function over the course of the disease. Although some medical groups have published information on the burden of ALS, these studies are small or geographically limited. The European Medicines Agency (EMA) is considering methods to better incorporate patient and caregiver input into regulatory review processes. Given the potential for EMA review of several new ALS therapies over the coming years, it is important for the community to develop this type of information. Objective: To conduct an on-line survey of European ALS patients and in-home caregivers to capture the burden of disease. The survey will generate information on patient burden across approximately 10 countries. There is anecdotal information and observations indicate that patients and their primary caregivers have different perceptions and concerns regarding the burden of disease. The survey will also capture how these perspectives change during ALS progression and what may be different among different patient demographics and subpopulations. The survey results will be analyzed in conjunction with the results of a survey carried out in the United States. Methods: A steering committee was established, consisting of industry partners, clinical and methodological experts, with input from patients and caregivers. Recruitment will be carried out with the partnership of European Network for the Cure of ALS (ENCALS) and advocacy groups in each country. A representative sample of patients and caregivers across disease severity, demographics and regional areas will be targeted. Conclusions: The ALS patient and caregiver survey in Europe will provide information on ALS disease burden and patient and caregiver perspectives into drug development processes.

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D29 Edaravone in amyotrophic lateral sclerosis: The experience of the former 6 months therapy in the neurological clinic of Pisa

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Edaravone is a molecule with antioxidant power, that showed to be capable of protecting endothelium and neuronal cells from high levels of oxidative stress in ischemic stroke and in animal models of Amyotrophic Lateral Sclerosis (ALS). On the 28th of June 2017, after the approval of Edaravone (Radicut/Radicava) in United States by Food & Drug Administration, AIFA (Agenzia Italiana del Farmaco) included it into the list of drugs delivered at the expense of the Italian National Health Service, in accordance with the law 23rd December 1996 n. 648, for the treatment of patients with definite or probable diagnosis of ALS. The specific inclusion criteria for the drug supply come from the Japanese phase III randomised double-blind placebo-controlled clinical trial conducted by the "Edaravone (MCI-186) ALS 19 Study Group" (Abe et al., 2017), that showed the evidence of a slowdown in the disease progression rate in patients with short disease history and mild disability. The treatment schedule consists in 28-days treatment cycles. During the first cycle, for the former 14 days, Edaravone is administrated intravenous everyday at the dosage of 60 mg, followed by 14 days of interruption; during the following cycles, for the former 2 weeks Edaravone is administrated at the same dosage for 5 days of both weeks, equally followed by 14 days of interruption. We discuss the results of the former 6 months therapy in 7 ALS patients followed at the Neurological Clinic of Pisa. As markers of the disease progression rate, we assessed clinical scores (ALSFRSr, ALSAQ5, 4 limbs MRC, I motor neuron involvement score, Forced Vital Capacity at spirometry) and biochemical serum markers of oxidative stress (AOPP, FRAP, thiols). As indicators of drug safety, we repetitively evaluated, by blood sampling, blood count, coagulation, hepatic and renal function, CPK. As results, all the markers assessed, both clinical and biochemical, were not significantly modified at the end of the last cycle of Edaravone administration. Two patients experienced severe adverse events. This protocol wasn't placebo controlled. However, if compared with data extracted from a previous clinical trial conducted in the Neurological Clinic of Pisa, in a larger ALS patients group, Edaravone seems to show a slight effect in decelerating the disease progression rate.

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D30 A post-hoc analysis of the edaravone phase III study 19: Regression analyses to examine long-term efficacy

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Background: The edaravone pivotal phase III study, Study 19 (MCI186-19), consisted of a 24 week placebo controlled period. At the end of 24 weeks, there was a 33% difference between edaravone and placebo (the change in ALS Functional Rating Scale (ALSFRS-R) score was -5·01 in the edaravone group and -7·50 in the placebo group in the primary analysis as planned). The placebo-controlled phase was followed by a 24 week open-label active treatment period to collect safety data. Objective: As the 24 week study extension was uncontrolled, we examined alternative methods to impute longer term effects of edaravone. Methods: Multiple linear regression analysis was used to develop a model to project the Study 19 placebo arm through cycle 12, to further assess the long-term efficacy of edaravone. Results: There were 68 patients in the treatment group and 66 in the placebo arm in the primary efficacy analysis of the Phase III study. At the end of cycle 12, the change in ALSFRS-R in the edaravone treated group projected via regression analysis was -8.61. The actual change in ALSFRS-R in edaravone was -8.02. At end of cycle 12, the change in ALSFRS-R in the placebo group projected via regression analysis was -13.03. Comparing projected placebo to actual edaravone at end of cycle 12, there is a 38.45% difference. Comparing projected placebo to projected edaravone at end of cycle 12, there is a 33.92% difference. Conclusion: These post-hoc findings, based on regression analysis, suggest that edaravone maintains efficacy up to 12 months which was the preplanned length of Study 19.

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D31 A post-hoc analysis of edaravone study 19: Forced vital capacity (FVC) subgroup analysis

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Background: Edaravone Study 19 (MCI186-19) employed a strategic study design in order to elucidate a treatment effect in a 6 month timeframe utilizing the ALS Functional Rating Scale- Revised (ALSFRS-R). Questions regarding the generalizability of the results to real-world utility (beyond study inclusion criteria, which included % Forced Vital Capacity (%FVC) > 80 continue to arise from both clinicians and payors. Objectives: To investigate the efficacy of edaravone over 6 and 12 cycles of treatment, as measured by ALSFRS-R, in patients who maintained a % FVC ≥ 80 through Cycle 6, compared to patients with an % FVC <80 at the end of Cycle 6. Methods: Using data from Study 19 (Cycles 1-6 randomized to edaravone or placebo; Cycles 7-12 open label active treatment extension), we retrospectively examined the change from baseline ALSFRS-R at the end of cycle 6 and cycle 12 in patients based on their FVC values at the end of cycle 6 (% FVC ≥ 80; % FVC 80 at the end of Cycle 12, the between group difference for edaravone to placebo was 23.0% [edaravone -7.38; placebo -9.58]. The change in ALSFRS-R from baseline in patients with a % FVC<80 at the end of cycle 6 was -5.16 in the edaravone group (n=25) and -9.1923 in the placebo group (n=26). There was a 43.9% difference between the groups at the end of cycle 6. For patients with a % FVC<80 at the end of Cycle 6, the between group difference in ALSFRS-R at 12 months was 31% [edaravone -9.71; placebo –14.09]. Conclusion: This analysis suggests that edaravone has benefit in ALS patients, despite experiencing a decline in their FVC.

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D32 Towards more efficient clinical trial designs in ALS: Lessons from the edaravone development program

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Background: Clinical trials in ALS have been challenging to design for a variety of reasons including: heterogeneity of symptoms, rate of progression, and limitations of assessment tools. The edaravone development program employed a strategic enrichment design to Study 19 (MCI186-19) to address these challenges, based on key learnings from the preceding Study 16, so that a treatment effect could be documented within a 6 month timeframe utilizing the ALS Functional Rating Scale- Revised (ALSFRS-R) as the primary endpoint. Objective: Using post-hoc assessments of Study 16 and Study 19, we examined how differences in study design, specifically inclusion criteria, influenced the ability to detect a treatment effect, as assessed by ALSFRS-R. Methods: In the current post-hoc analyses of Study 16 and Study-19, a decrease in the ALSFRS-R score was examined for the placebo controlled cycles 1-6. Patients' disease progression based on ALSFRS-R changes over the course of the 24 weeks study was defined as: No Progression = zero point decline, Minimal Progression ≤ 2 point decline, Slow Progression≤ 5 point decline, and Significant Progression >9 point decline. Results: Study 16 had 205 patients in the full analysis set (FAS; 102 edaravone, 104 placebo) Study 19 had 137 patients in the FAS (68 edaravone, 69 placebo). In placebo patients in Study 16, 18% experienced no progression, 36% minimal progression, 64% were slow progressors and 25% experienced significant progression. In Study 19, 6 % experienced no progression, 13% minimal functional decline, 51% were slow progressors and 24% experienced a significant functional decline Conclusion: It is likely that the study enrichment that maximizes the propensity for dynamic change (and thus, the potential for an experimental therapy to modify that change) is important for efficient clinical trial design. These post-hoc findings suggest that Study 16 included a large proportion of slow progressors, over 1/3 of the placebo group deteriorated an average of 0.13 points per month. This therefore, reduced the ability to detect a treatment effect for a drug which slows progression. By applying these learnings to Study 19 design, investigators were able to decrease the number of slow progressors and enhance the ability to show the effects of edaravone treatment on disease progression.

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D33 A phase 2, double-blind, randomized, placebo-controlled, multiple-dose study of reldesemtiv in patients with ALS (FORTITUDE-ALS)

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Background: Reldesemtiv, also known as CK-2127107, is a selective small molecule fast skeletal muscle troponin activator (FSTA) that sensitizes the sarcomere to Ca2+ by slowing the rate of Ca2+ release from troponin. In a force-frequency study in healthy volunteers, reldesemtiv increased the force generated by the tibialis anterior muscle versus placebo in response to nerve stimulation in a dose-, plasma concentration-, and frequency-dependent manner. Reldesemtiv showed a greater pharmacodynamic effect at lower plasma concentrations than did tirasemtiv, which is also an FSTA but of an unrelated chemical structural class. Reldesemtiv was designed to minimize crossing of the blood brain barrier and is not known to inhibit cytochrome P450 isozyme activity. Inadequate tolerability to tirasemtiv, largely due to CNS adverse effects such as dizziness, led to increased numbers of drop-outs in participants receiving tirasemtiv in the phase 3 clinical trial, VITALITY-ALS (also known as CY 4031). Reldesemtiv appeared to be better tolerated than tirasemtiv in healthy volunteers, providing a rationale for its study in participants with ALS. Methods: Key entry criteria for FORTITUDE-ALS (also known as CY 5022) include slow vital capacity (SVC) >65% predicted and an ALS diagnosis within 24 months. Participants are randomized 1:1:1:1 to placebo or reldesemtiv at 150 mg bid, 300 mg bid, or 450 mg bid for 12 weeks. The primary endpoint is the change from baseline in SVC at 12 weeks. Exploratory home-based outcome measures are included in this study to evaluate their utility. Weekly at home, participants will perform voice recording on a mobile device and SVC measurements on a portable spirometer. Fine motor skills are assessed at clinic visits using an iPad application. Safety, pharmacokinetic (PK) and traditional ALS outcome measures, such as mega-score of muscle strength and ALS Functional Rating Scale â€" Revised, are also assessed in the clinic during the trial. Conclusions: Based on favorable safety, PK, and pharmacodynamic findings of reldesemtiv in its Phase 1 studies, FORTITUDE-ALS was designed and is currently being conducted in the USA and Canada, and is planned in Australia and certain European countries (Ireland, Spain, and the Netherlands).

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D34 A single-blind, randomized controlled clinical trial to evaluate the effects of intensive motor rehabilitation in ALS patients (ERMOSla)

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Amyotrophic lateral sclerosis is a relentless progressive neurodegenerative disease. In the lack of a disease-modifying treatment, many advancements were made in the field of patients' clinical management and in the multidisciplinary approach that result in life prolongation for ALS patients. During the disease course, the spread of muscle atrophy and the cardiorespiratory impairment lead to inactivity and to forced postures with subsequent pain and contractures, as well as psychological drawbacks to patients and their caregivers. To avoid this and in the setting of the multidisciplinary care, physical exercises are offered to patients by physiotherapists. Despite these achievements, there are not clear evidences about the type of exercise, the regimen and which outcome variables have to be routinely assessed. Moreover, there are only few randomized controlled studies evaluating the effect of motor rehabilitation on ALS patients with consequent lack of robust and consistent results. We carried out a multicentre, single-blind, randomized, controlled study in order to compare the effects of the intensive exercise regimen (IER) made of 5 sessions/week to the usual exercise regimen (UER) of 2 sessions/week during a 10 weeks-long study period. In the next 24 months-long follow-up period, all patients underwent the usual exercise regimen (UER) twice/week. Each exercise session was 45 minutes long and it was made of a mixture of aerobic, endurance and low-load resistive training associated with stretching of retracted muscles. The primary outcome of the study was quantification of overall disability as measured by change in ALS-FRS-R scale. Three Italian ALS multidisciplinary centres took part in this study. Between July 2013 and July 2017 we enrolled 33 patients for each treatment group with a definite, probable or possible diagnosis of ALS, a clinical onset within 18 months and aged between 18 and 86 years; main exclusion criteria were cognitive impairment, cardio-respiratory diseases or other unstable clinical conditions. An evaluating neurologist blinded to the allocation group visited the patients and performed the scales every month for the first six months and then every three months. We showed no significant difference with respect to the primary outcome (ALS-FRS-R decline) between the IER and the UER group.

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D35 Neuregulin 1 Type III gene therapy improves SOD1-linked amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a devastating motoneuron disease with no effective cure currently available. Molecular mechanisms that are specifically involved in the death of motoneurons are still not fully uderstood, and in addition neighboring cells such as microglia, astrocytes and interneurons directly contribute to the disease. Neuregulin 1 (NRG1) is a trophic factor highly expressed in motoneurons and neuromuscular junctions. Recent evidence suggests that NRG1 and their ErbB receptors are involved in ALS. However, the role of the NRG1-ErbB pathway on motoneuron survival is still controversial. Due to the low protein levels of NRG1 Type III in the central nervous system of ALS patients here we directed gene therapy based on adenoassociated viruses to overexpress NRG1 Type III on the central nervous system in SOD1G93A ALS mice. The mice were evaluated from 8 to 16 weeks of age by means of nerve conduction and rotarod tests. At 16 weeks they were sacrificed for histological analyses. Our results indicate that over-expression of NRG1 Type III is able to preserve motor function of tibialis anteriors and gastrocnemius muscles, improve the locomotor performance, maintain the number of surviving motoneurons at 16 weeks, and reduce glial reactivity in the treated SOD1G93A mice. These findings indicate that increasing NRG1 Type III at the spinal cord is an interesting approach for promoting motoneuron protection in Amyotrophic Lateral Sclerosis.

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D36 Exploring the proteome of ALS laser microdissected Purkinje cells: Method development

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Liquid Chromatography-Mass Spectrometry (LC-MS/MS) sensitivity has improved significantly over the last decade and despite these developments, it has had a low impact in the field of neuroscience compared to other techniques such as MRI, in-situ hybridisation and optogenetics. This is partially due to the heterogeneous nature of the human brain which makes acquiring proteomic data from single cell populations challenging. The human central nervous system is extremely heterogeneous, it contains 100 billion neurons with several 100 trillion interconnections; collectively they perform a plethora of functions including cognition, sensory and motor control. All functions of the brain are ultimately mediated by proteins and exploring the proteome of individual cells can reveal additional functions and responds to neurodegenerative diseases. However, it is often difficult to extract single-cell proteomic data from the CNS as a single biopsy can contain hundreds of cell types which can mask individual proteomes. A targeted single-cell proteomic approach needs to be utilised to explore the pathology of individual cells. Our group has developed a 'microproteomic' method using Laser Capture Microdissection (LCM) to isolate single cell populations and analyse their proteins with LC-MS/MS. We have developed a workflow optimising sample dissection, histology, LCM, sample preparation, protein reduction and alkylation and LC-MS/MS analysis through our collaboration with the Target Discovery Institute. Using this approach we have increased the proteomic coverage and depth of our samples to a resolution comparable to bulk tissue proteomics, using an input of only 200 cells. We have obtained a dataset of ~2,500 proteins from individually microdissected Purkinje Cells from a post-mortem brain with Amyotrophic Lateral Sclerosis. The proteome of these Purkinje cells was analysed against control tissue. We found an enrichment of proteins relating to mRNA processing (p 1.29E-03), mRNA splicing (p 3.85E-02) and an increase in RNA Polymerase II Transcription (p 9.53E-03) and RNA Polymerase II Regulation (p 1.71E-02). We also see a 6-fold enrichment in proteins that epigenetically regulate gene expression (p 3.20E-03). Our microproteomic approach is now being used to explore the differing proteomes of individual cells in ALS. This technique allows cells that were previously thought too difficult to remove from their cellular environment to be researched with higher sensitivity.

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D37 Tract pathology in amyotrophic lateral sclerosis correlates with aggressiveness of disease as defined by the D50 progression model

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Background: Detecting the progression of ALS-related neurodegeneration is essential for clinical trials and facilitates early diagnosis. Hence, there is an urgent need to identify ALS caused changes in the brain in different ALS subtypes. The aim of this study is to correlate Diffusion Tensor Imaging (DTI) structural changes in neuronal fiber tracts with parameters for the time-course of ALS disease progression, as ascertained from the D50 model (1,2). Methods: DTI scans of 59 healthy controls and 82 ALS patients from a 1.5 T scanner were analyzed using the FSL package, including brain-extraction, eddy-current-correction and tensor decomposition. FA and MD maps were used for Tract-Based Spatial Statistics (TBSS). Inter-group contrasts were calculated between patients and controls, corrected for age as confounding covariate. The D50 model was used for TBSS subgroup- and regression analyses. Referring to D50 (time until ALSFRS-R drops to 24) the ALS cohort was subdivided into fast (D50 < 30 months, n= 38) and slow progressors (D50 â‰¥ 30 months, n = 44). Correlations between FA or MD values and the calculated functional status at the time of MRI acquisition (FSMRI) were examined in a regression analysis. Results: TBSS inter-group-comparison revealed ALS-related widespread white-matter pathology, as indicated by decreased FA and increased MD in multiple supra- and infratentorial tracts. Subgroup-analyses showed higher MD values for fast progressors in bihemispheric motor- as well as non-motor related pathways. Lower FA values were found in the left corticospinal-tract and frontal pathways in contrast to the slow-progressors. In a regression analysis increased MD values were correlated with a lower functional status at the day of MRI acquisition (calculated by the D50-model as FSMRI) mainly in interhemispheric fibre tracts and dominated by the right hemisphere. Conclusion: Using DTI-based TBSS we were able to identify structural changes in the white-matter-network of ALS patients that correlated with clinical progression parameters. Increased MD related to fast ALS-progression was revealed in many fibre tracts, emphasizing the view of ALS as multisystemic neurodegenerative disorder whose aggressiveness can be detected using MRI. 1 Poesen et al., Neurology. 2017; 88(24):2302-2309 2 Gaur et al., ALSFTD Journal 2017, Boston conference poster

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D38 ALS Cell Atlas: An online resource to infer gene activity in nine major CNS cell types in ALS patients and mouse models

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We still know very little about how various central nervous system (CNS) cell types contribute to ALS onset or progression. Our goal is to infer the timecourse of activity for nine major CNS cell types in spinal cord tissue from end-stage ALS patients and during disease progression in SOD1G93A mouse model with the use of single-cell transcriptomes applied to interpret whole tissue expression data. The resulting dataset will be released as an open, interactive online resource. Our resource will include gene expression specificity rankings for nine major cell types (pyramidal neurons, interneurons, astrocytes, microglia, oligodendrocytes, endothelium, pericytes, vascular smooth muscle and vascular leptomeningeal cells). In order to observe the dynamics of cell activity we analyzed respective cells at pre-symptomatic (28, 42, 56, 70 days) and symptomatic stages (98, 112, 128 days) in SOD1G93A mice together with post mortem ALS patient tissue. We would like to present a demonstration version of the online resource features for genes associated with microglial cells which allowed us to identify novel genes activated during ALS in these cells. Our resource will help to stimulate interest in specific cell type contribution to ALS. It will also help to discover novel drug targets specific for given cell type population in symptomatic and pre-symptomatic stages of ALS progression. In the long run we hope our resource will stimulate drug discovery research and benefit ALS patients.

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