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Poster List

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Posterlist ENCALS meeting 2023

Basic research

1. 3D in vitro brain and spinal cord cell models to identify early neuronal vulnerability and test therapies in C9ORF72-Amyotrophic Lateral Sclerosis

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Introduction

Amyotrophic lateral sclerosis (ALS) is an untreatable neurodegenerative disorder whose pathogenic mechanisms are still incompletely understood. C9ORF72, whose hexanucleotide repeat expansion (HRE) represents the main genetic cause of ALS, has been postulated to play a role in neurodevelopment. To investigate whether early developmental vulnerability in ALS could result in late onset neurodegeneration, we will exploit 3D patient-specific in vitro models of central nervous system (CNS).

Methods

We generated induced Pluripotent Stem Cell (iPSC)-derived brain (BrOs) and spinal cord (ScOs) organoids of C9ORF72-ALS patients and isogenic controls, using a free-floating, 3D-culture method, based on EBs aggregation, matrigel embedment and agitation in spinning bioreactor. Structural and functional characterization was performed. Organoids were treated with an antisense oligonucleotide (ASO) targeting C9ORF72-HRE.

Results

BrOs and ScOs expressed pluripotency markers and mature neuronal markers in early and late stages, respectively. C9ORF72-ALS organoids presented higher rate of cell death and a lower degree of maturity compared to isogenic controls. C9ORF72-ALS organoids recapitulated disease hallmarks and displayed disrupted key cellular processes, like DNA and axonal damage. BrOs and ScOs are functionally active at calcium imaging recording but behave differently after glutamate stimulation, suggesting a different neuronal excitability in ALS. Treatment with C9ORF72-directed ASO was effective in ameliorating disease phenotype.

Conclusion

Patient-specific iPSC-derived 3D CNS models reproduce at different time points the maturation of neural and glial cells, resembling physiologic human neurodevelopment. BrOs and ScOs are valuable tools for disease modeling since they improve the characterization of C9ORF72-ALS pathology, dissecting specific disease hallmarks, and provide the opportunity to test therapeutic strategies.

2. A Novel Gene Therapy Approach for ALS by Overexpressing the Pleiotropic Chronokine α-Klotho

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In Amyotrophic lateral sclerosis (ALS), muscle denervation and degeneration of motoneurons (MNs) result in progressive muscle weakness and atrophy. Preventing axonal detachment from muscles, protecting MNs and promoting reinnervation are key to improve the functional outcome of ALS.

α-Klotho is a pleiotropic chronokine with an excellent profile as neuroprotective and myoregenerative agent by means of anti-oxidative and anti-inflammatory properties, promoting myelination, protecting from excitotoxicity, and maintaining mitochondrial ultrastructure and function. In the SOD1G93A mouse model, which recapitulates most of ALS abnormalities, we have found decreased mRNA levels of α-Klotho in skeletal muscles, motor cortex and lumbar spinal cord. Furthermore, in rat spinal cord organotypic cultures, the overexpression of α-Klotho protects spinal MNs from glutamate-induced excitotoxicity.

Given the pleiotropic beneficial properties of α-Klotho, we hypothesized that boosting its expression and secretion in skeletal muscles through a one-time gene therapy treatment would protect muscles from atrophy and prevent axonal retraction and neuronal loss in SOD1G93A mice. Secretion of α-Klotho by muscles, mediated by a myotropic AAV vector, enhances motor function and the strength of the animals and delays the onset of the disease. Neuromuscular functional improvement was reflected as increased compound muscle action potential (CMAP) amplitudes and by larger size and number of functional motor units of hindlimb muscles compared to mock-treated controls. α-Klotho-treated SOD1G93A mice show more surviving motoneurons and a significant reduction in microglial and astroglial reactivity in the ventral horn of the spinal cord. Increased amplitude of the motor evoked potentials (MEPs) also indicates the preservation of central connectivity between upper and lower MNs. All this correlates with a higher number of innervated motor endplates and a preserved mass of the muscles.

Overall, our results provide evidence that the secretion of α-Klotho in muscles can promote functional improvement in ALS and may open a new avenue for the treatment of this devastating disorder.

3. A repurposing approach to improve disease outcome in SOD1G93A mice by counteracting oligodendrocyte dysfunction and neuroinflammation

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive loss of motor neurons (MN) and consequent muscle atrophy, for which no effective therapies are currently available. Recent findings revealed an early role of oligodendrocyte (OL) dysfunction in promoting MN degeneration and disease progression. On this basis, restoring proper myelination and trophic support to MNs by fostering oligodendrocyte precursor cell (OPC) maturation may open new therapeutic perspectives for ALS. An important regulator of OPC differentiation is the P2Y-like GPR17 receptor, which drives the initial steps of this process, and it is then downregulated to allow OPC maturation. Our previous results revealed that an abnormal increase of GPR17 expression is associated to OL dysfunction in the spinal cord of the SOD1G93A murine model of ALS. Accordingly, primary OPCs isolated from SOD1G93A mice displayed differentiation defects compared to wild-type cells, which were rescued by in vitro exposure to the non-selective GPR17 antagonist montelukast (MTK). Overall, these results suggest that the repurposing of MTK, an already marketed and safe anti-asthmatic drug, may represent a promising therapeutic strategy in ALS.

Here, we evaluated in vivo the effects of the oral administration of MTK (30 mg/kg/day), from early symptomatic phase until end stage, in male and female SOD1G93A mice compared to vehicle-treated littermates. MTK treatment was found to significantly increase survival probability, delay body weight loss, and ameliorate motor functionality of female SOD1G93A mice, while no effects were observed in males. Noteworthy, immunohistochemical analysis revealed that MTK administration significantly counteracted the pathological GPR17 upregulation, restoring the number of CC1+ mature cells in the spinal cord of female SOD1G93A mice. In addition, in the same tissues, MTK treatment was found to enhance the regenerative properties of microglia and astrocytes, limiting their detrimental reactive state, and resulting in improved MN survival. Highly relevant to a translational perspective of these results, immunohistochemical analyses showed an increased density of OLs expressing GPR17 in post-mortem spinal cord tissues from ALS cases with respect to control subjects. Globally, these data support the relevance of a GPR17-based pharmacological approach for ALS treatment.

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4. Activation of Boron transporter (NaBC1) in muscle generates neuroprotection in a SOD1 ALS mouse model.

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This work is based on the functional coupling of integrins, boron (B) transporter (NaBC1) and growth factor receptors (GFRs) after their activation. In our previous work, we have demonstrated that active-NaBC1, co-localise with integrins and GFRs producing a functional cluster that synergistically enhances biochemical signals and crosstalk mechanisms, accelerating muscle repair after an injury and restoring dystrophic phenotypes in vivo in muscular dystrophies with different aetiological origin. Thus, we aimed to study the effects of B in ALS mouse model targeting muscle. We have engineered and characterised injectable alginate-based hydrogels with controlled local B-release. Selected compositions were injected in both quadriceps at 10, 12, 14 and 16 weeks old B6SJL-Tg(SOD1-G93A)1Gur/J ALS male and female mice randomly distributed between different groups. We have followed changes in body weight and motor impairment in behavioural four limb hanging test along 12 weeks. Results showed increased body weight and hang time of the B-treated mice compared with non-treated control group. After euthanasia at 17 weeks old, different tissues were analysed by histological and immunofluorescence techniques. The histoarquitecture of quadriceps muscle from B-treated mice, although still displaying ALS pathology, resulted in a significant decrease in size variability among muscle fibres. The evaluation of muscle pathology by analysing muscle inflammatory mast cell recruitment and IL-6 revealed that B-treated mice presented a strong decrease in inflammatory markers. Immunofluorescence analysis showed that B treatment significantly decreased muscle type 1 slow myofibers, suggesting the prevention of the loss of type 2 fast myofibers, which drastically decrease during ALS. Further, Pax7+ cells were significantly increased in ALS B-treated mice compared with control, indicating a major number of active satellite cells involved in muscle regeneration and repair. Analysis of spinal cord and brain showed an increase in motor neurons and white substance in treated mice as well as decrease in GFAP marker indicative of glial reactivity. Altogether, our findings show that active-NaBC1 promotes ALS muscle repair and prevents muscle and nerve inflammation, suggesting a retrograde neuroprotection effect from the muscle treatment and representing a novel mode of action to explore new therapies for ALS treatment.

5. ALS is associated to disorders of riboregulation of p62 by vtRNAs

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In recent years, defective RNA regulation has been associated with amyotrophic lateral sclerosis (ALS). Vault RNAs (vtRNAs) are small RNAs that offer a novel layer for controlling autophagy, the so-called riboregulation. These RNAs interact with p62-SQSTM1, which regulates its polymerization. As p62 bodies are often present in ALS and surrogate models, we hypothesized that vtRNA might be altered in this context. Using RT-qPCR, we set up a quantitative analysis of vtRNA levels for murine and human-origin samples. To benchmark this system, we evaluated the expression of these vtRNAs in a cellular model of regulable TDP-43 expression, demonstrating that TDP-43 controls the levels of vtRNA. Although the effect of Tdp-43 transgenesis in the expression of the mouse vtRNA ortholog was not as robust, there was a trend towards increased expression. In lumbar spinal cord samples from other ALS-like models (G93A mice), we observed a statistically significant increase in the mouse vtRNA levels in an age-dependent mode. Interestingly, we observed a decrease in the levels of a group of vtRNAs (1-1, 1-2, and 1-3 present in the same locus) in spinal cord samples from sporadic ALS patients but not in the levels of vtRNA2-1 (located in a different locus), which has been recently overexpressed in other neurodegenerative conditions. Globally, our findings are compatible with TDP-43 loss, impinging a decrease in vtRNAs and thereby contributing to the buildup of p62 bodies and autophagy disorders in ALS.

6. ALS/FTD-associated C9orf72 C4G2 repeat RNA disrupts phenylalanine tRNA aminoacylation and protein production

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The most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the C9orf72 gene mutation, which results in expanded hexanuleotide repeat – GGGGCC. It transcribes in sense (G4C2)n and antisense (C4G2)n direction and leads to the formation of nuclear RNA foci. We have identified proteins that bind to antisense transcripts. These include proteins involved in protein synthesis, cytoskeleton stability and mRNA processing. We performed RNA-pull down assay from mouse and human brain lysates followed by mass spectrometry to determine protein interactors and validated them using WB, FISH/ICC. For the first time we observed the interaction with phenylalanine-tRNA synthetase (FARS). We developed a modified RNA-protein proximity ligation assay to observe cytoplasmic interactions of the repeats, since up until now FISH method only detected nuclear interactions.

To evaluate the impact of antisense RNA-FARS interaction on tRNA aminoacylation, protein synthesis and cellular stress we used two aminoacylation assays, western blot analysis and Click-chemistry. Antisense RNA-FARS interaction resulted in significant decrease in aminoacylation rate in in vitro assay and lower charged tRNAphe levels in patient derived lymphoblasts compared to controls. Additionally, we observed a decreased expression of phenylalanine-containing proteins at the whole protein level using modified click-chemistry and of four individual proteins with high phenylalanine content. We also evaluated the effect of the lower aminoacylation level on cellular stress. In the presented study we investigated and confirmed protein interactions with the biologically relevant 32×C4G2 RNA repeats and how this affects cellular processes. Our discovery highlights the role of aminoacyl-tRNA synthetases in C9orf72 ALS/FTD where they may be important contributors to the development of these diseases. Other studies have previously linked irregularities in aminoacyl-tRNA synthetases to other neurodegenerative disorders.

7. ALS-associated GLT8D1 mutations disrupt membrane lipid rafts and localised neurotrophin signalling

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Background:

Mutations within GLT8D1 are associated with amyotrophic lateral sclerosis (ALS). Pathogenic mutations such as p.R92C impair GLT8D1 glycosyltransferase enzymatic function via a dominant negative mechanism, yet the downstream mechanism leading to neurotoxicity is unclear. Glycosyltransferase function has been linked to membrane lipid rafts (MLRs), which are disrupted in ALS. An important constituent of MLRs is the scaffolding protein caveolin-1 (CAV1). We previously identified ALS-associated mutations within enhancers linked to the expression of CAV1/CAV2 that reduce CAV1 expression and disrupt MLRs. In neurons, MLRs form an organising centre for neurotrophin signalling. We explore whether dysfunction caused by GLT8D1 mutations converges on disruption of MLR structure and function, and downstream neurotrophin signalling.

Methods:

We induced stable expression of p.R92C-GLT8D1 in isogenic HEK293 cells and evaluated changes in Golgi morphology and membrane ganglioside expression via confocal microscopy. We confirmed our findings in an ALS-relevant model by overexpressing p.R92C-GLT8D1 in mouse primary neurons via lentiviral transduction. We subsequently evaluated TrkB expression within MLRs isolated by sucrose-density fractionation.

Results:

We show that p.R92C causes fragmentation of the Golgi network and reduces ganglioside expression within MLRs, leading to impaired neurotrophin signalling. Expression of p.R92C-GLT8D1 in HEK293 cells and mouse primary neurons reduces expression of GM1 gangliosides within the cell plasma membrane leading to disruption of MLRs. Furthermore, overexpression of p.R92C-GLT8D1 within primary neurons reduces TrkB receptor expression within MLRs. In contrast, up-regulation of wild-type GLT8D1 enhances both MLR formation and accompanying TrkB expression.

Conclusion:

Our results closely mirror findings for another ALS risk gene, CAV1, suggesting convergence on a common pathogenic pathway. Other ALS genes have been associated with Golgi dysfunction and may disrupt the same pathway. We suggest that upregulation of GLT8D1 is a potential new therapeutic target for ALS.

8. Altered dynamics of DNA damage repair associated proteins in a human, neuronal in-vitro cell model of FUS-ALS

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Background:

Mutations in FUS (Fused in sarcoma) are causally associated with about 5% of genetic amyotrophic lateral sclerosis (ALS) cases. FUS is predominantly located in the cell nucleus and functions as a DNA/RNA-binding protein in splicing, transcription, stress response and DNA damage repair (DDR). As a result of DNA damage, PARP1 (poly-(ADP-ribose) -polymerase 1) is primarily activated, which synthesizes branched PAR chains to which FUS and other proteins of the DNA repair apparatus are recruited. Recently, the insufficient recruitment of mutant FUS to the site of DNA damage was identified as an important pathophysiological upstream event of neuronal demise.

Methods:

Human, spinal motoneurons derived from patient specific induced pluripotent stem cells were used as the primary cell model of this study. CRISPR/Cas9n edited cell lines with either WT-FUS-GFP or FUS-P525L-GFP were irradiated by laser ablation microscopy using a 355nm UV laser and subjected to indirect immunofluorescence to visualize the individual DDR factors.

Results:

Upon laser irradiation, FUS-P525L-GFP showed significantly less recruitment to the DNA damage site compared to the wild type. Repeated measurements of the cytoplasmic FUS-GFP signal over time demonstrated that FUS mutants are nonetheless able to recruit from the cytoplasmic FUS reservoir in order to ensure a slight nuclear accumulation after DNA damage induction, whereas in WT neurons the sole nuclear abundancy of FUS was sufficient for recruitment.

Pharmacological inhibition of PARP1 induced a loss of FUS recruitment in either genotype after 30min of treatment. Interestingly, the synthesis of PAR chains at the DNA damage site significantly decreased only after 24h treatment after PARP1 inhibition. However, the synthesis of PAR in general was significantly higher in WT neurons.

\Regarding DDR proteins downstream of FUS, there was a significantly reduced recruitment for XRCC1, KU70 and KU80 in FUS mutant neurons.

Conclusions:

The signalling pathways of both the classic DNA double-strand break repair and the single-strand break repair were negatively affected by the c-terminal FUS mutation P525L. Due to the fact that the mutation leads to a nuclear deficiency of FUS, it can be assumed that this ALS-associated protein has a universal function in DNA damage repair.

The observed lack in PAR after damage induction might be due to deficits in the ATP reservoir in mutant neurons

9. An electrophysiological study on C9orf72 associated motor neuron disease

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Monogenetic forms of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's Disease, account for about 10% of all cases. The most common genetic mutation is the intronic GGGGCC-hexanucleotide repeat expansion in the C9orf72 gene, which translates into toxic dipeptide repeat proteins (DPRs). Our previous findings suggest Sirtuin-1, a NAD+-dependent histone deacetylase, as a common denominator in DPR-induced pathway dysregulation. Sirtuin-1 has been shown to play a crucial role in neurodegeneration, as its dysfunction propagates p53-induced apoptosis, inflammation, and TDP-43 accumulation. Our preliminary results are indicative of Sirtuin-1 decrease in C9-ALS, induced by DPR toxicity, as well as selective TDP-43 accumulation in areas of post-mortem brain tissue which topographically correspond to the clinical manifestation of ALS. However, it is not yet understood what contributes to this selective degeneration of upper and lower motor neurons.

Metabolic disturbance has been reported to play a critical role in ALS progression, for example shown by a defective expression of tricarboxylic acid (TCA) cycle intermediates or mitochondrial dysfunction in the central nervous system. This is supported by the role of Sirtuin-1 as a modulator of metabolism and its ability to sustain cellular homeostasis by detecting energy deficits. It has been suggested that the continuous metabolic deficit not only accelerates disease progression but may also facilitate dysregulation of metabolically sensitive potassium channels and consequently cause selective hyperexcitability and degeneration of motor neurons.

We therefore aim to investigate the role of Sirtuin-1 downregulation on the electrophysiological properties of patient derived induced motor neurons. Furthermore, we will study the role of hyperexcitability in motor neuron degeneration in ALS.

Conflict of interest

The authors declare no conflict of interest related to this work

10. An homozygous C9orf72 transgenic mouse model displays enhanced molecular hallmarks of ALS and reveals AS gene therapy ability to target these traits.

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by a progressive loss of upper and lower motor neurons, leading to muscle atrophy. Mutations in several genes have been identified as causative of this disease, among which the G4C2 hexanucleotide repeat expansion (HRE) in the first intron of the C9orf72 (C9) gene, responsible of the majority of ALS cases. Three main pathological mechanisms have been linked to this mutation: a decrease in the expression of C9 protein, an accumulation of sense and antisense RNA foci and the production of toxic dipeptide repeats (DPR). To date there's no animal model of C9-ALS that fully recapitulates the key disease symptoms. One of the available models for preclinical studies is the C9 hemizygous (He) transgenic mouse expressing a human C9 gene with 500 HREa. This model was described as presenting acute progressive disease in 30% of the female mice, however the phenotype was not reproduced in some studies and in our hands. The lack of an ALS phenotype in the C9 He mice pushed us to generate and characterize for the first time the homozygous (Ho) C9 mouse model, expected to exhibit an emphasized disease progression. The new colony was asymptomatic, as the He mouse, but showed a higher accumulation of sense (+32%) and antisense (+75%) RNA foci and DPR in the brain (+30%) and in the spinal cord (+50%) compared to the He. We therefore decided to use the Ho model to test our therapeutic approach for C9-ALS, based on antisense sequences (AS) targeting the first intron of C9 pre-mRNA, expressed by U7 small nuclear RNA in a self-complementary (sc) adeno-associated viral vector, serotype 10 (scAAV10-U7-AS). As a proof of concept, we treated neonate C9 female mice by intracerebroventricular injection of 6 different scAAV10-U7-AS and followed them for 3 months. We identified one AS which improves the molecular features in Ho C9 mice. We next treated adult Ho mice at 3 months of age, when the molecular hallmarks are already present, and we confirmed what observed in the treated new-born mice: a 50% decrease in HRE containing C9 mRNA, a 40% decrease in sense and antisense RNA foci as well as a 60% reduction of DPR accumulation. To conclude, our study shows that Ho C9 mice display a higher accumulation of molecular features of C9-ALS than He mice, and that gene therapy using scAAV10-U7-AS allows a rescue of the molecular phenotype in both newborn and adult animals.

11. An in vitro investigation of cortical network hyperexcitability in mouse models of ALS

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Amyotrophic lateral sclerosis is an incurable neurodegenerative disease that leads to the progressive death of cortical and spinal motor neurons. Growing evidence indicates that cortical hyperexcitability, the earliest detectable neurophysiological change in pre-symptomatic ALS, is a strong candidate for driving excitotoxic cascades and subsequent cortical and motor neuron degeneration. Intriguingly, neurophysiological disturbances are early pathogenic features of all ALS patients and thus attractive therapeutic targets, however the underlying mechanisms are not well understood. Multi-electrode arrays combined with in vitro dissociated cortical neuron cultures provide a powerful platform to decipher mechanisms which drive excitability in transgenic mouse models of ALS and the discovery of novel therapeutics. In this study, we perform an in-depth investigation of hyperexcitability in cortical neuronal networks of different ALS mouse models. We reveal that hyperexcitability profiles are found only in models displaying motor system degeneration. Specifically, we found early network excitability alterations in G93A-SOD1 derived cortical neurons, that were evidenced by increased bursting frequency compared to control neurons. Pharmacological examination of the G93A-SOD1 network uncovered that cortical hyperexcitability was accompanied by inhibitory signaling deficits, but not changes in the excitatory input. Furthermore, we showed absence of hyperexcitability phenotype in cortical neurons derived from two different C9ORF72-BAC mouse models which show molecular phenotypes only. Our results report early excitability changes in G93A-SOD1, but not in C9ORF72-BAC, cortical neurons, confirming that cortical hyperexcitability is only observed in models which go on to display a motor phenotype associated with motor neuron loss.

12. Analysis of stress response in Amyotrophic Lateral Sclerosis (ALS) patient fibroblasts

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Proposed pathogenetic mechanisms for Amyotrophic Lateral Sclerosis (ALS) motor neuron disease include abnormal intracellular trafficking on cytoskeleton networks, protein misfolding/ accumulation and RNA metabolism dysregulation. The identification, in some ALS patients, of mutations in genes encoding components of stress granules (SGs), transiently formed to preserve mRNA during cellular stress, supported that inappropriate response to stress could be part of the neurodegenerative process. In this study we compared the stress response in control and ALS patient fibroblasts following sodium arsenite exposure. We observed a common phenotype in ALS fibroblasts, combining a delay in SG disassembly, persistence of cytoplasmic protein inclusions containing TIA1 and G3BP1 (stress granule markers) with partial colocalization of TDP-43 and p62 (two well-known components of the protein deposits that are characteristically observed in ALS patient post-mortem spinal cord tissues), and decreased cell viability after stress recovery. Such defects, detectable in cells normally not thought to be affected by degeneration in ALS, such as fibroblasts, could result from an initial TIA1 “cellular scar”, persisting after cellular stress in fibroblasts of ALS patients, that may drive a common early mechanism of cellular dysfunction, leading - in motor neurons - to their subsequent degeneration. These observations suggest that fibroblasts could be used as a simple tool to study some of the ALS pathological processes.

13. Antibody based treatment: Amelioration of motor and cognitive performances and reduction of TDP-43 proteinopathy in mice models of ALS induced by the

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The majority of ALS cases (~90%) are considered sporadic (sALS) and their etiology still remains unknown. However, many studies suggest that TDP-43 could play a role in neurodegeneration. Indeed, abnormal cytoplasmic aggregates of TDP-43 (TAR DNA-binding protein 43) are a pathological hallmark of degenerating neurons in many neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), Alzheimer's disease and Parkinson's disease, for which there is currently no effective treatments. To target TDP-43 and reduce the toxicity due to its accumulation and aggregation, we tested an antibody (E6) directed against the RRM1 (RNA Recognition Motif 1) domain of TDP-43. Previously, our lab (Pozzi et al.,2019; Pozzi et al., 2020) reported that this antibody can be used to mitigate TDP-43 pathology in transgenic mice expressing mutant TDP-43. Moreover, our lab (Mishra et al., 2020) reported that intracerebroventricular (ICV) infusion of ALS patients CSF in mice expressing the human wild type TDP-43 protein (hTDP-43WT) induces an ALS-like phenotype. It is documented that removal of misfolded SOD1 by C4F6 antibody using immunoprecipitation reduces the toxicity of ALS-CSF samples toward motor neuron-like cultured cells (Tokuda et al. 2019).

Here, we propose to test the antibody effect in mitigating the pathology and motor and cognitive deficits in a mouse models of ALS induced by ALS patients CSF intracerebroventricular (ICV) infusion. We tested to administration methods: intrathecal (IT) injections and ICV infusion along with the CSF. IT treatment led to amelioration of motor performance and reduction of TDP-43 proteinopathy in the lumbar spinal cord while ICV treatment led to amelioration of both motor and cognitive performances, as well as reduction of TDP-43 proteinopathy in the brain motor cortex of E6 treated mice compared to control. In a second aim, we propose to prepare, via immunocapture, ALS-CSF samples depleted of TDP-43 or misfolded SOD1. The intact and modified ALS-CSF samples will be infused ICV in mice co-expressing hTDP-43WT and hSOD1WT to investigate if CSF-induced pathology is alleviated. We expect that ALS-CSF samples depleted in TDP-43 or SOD1 will have reduced toxicity compared to intact ALS-CSF samples. These studies will provide new insights on disease mechanisms for sALS and will serve to advance the development of new treatments to halt disease propagation.

14. Assessment of protein aggregation in a model of ALS and cells derived from patients

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Amyotrophic Lateral Sclerosis (ALS) is a spectrum of disease in which both the diagnosis and treatment is intricate due to the heterogeneity between patients and the lack of biomarkers. [1, 2] Current experimental models do not reflect on this diversity, therefore a model consisting of samples extracted from patients is essential to characterize the pathology. Considering the degeneration in ALS is multisystem, an analysis of lymphoblasts from blood samples is proposed. [3]

One of the most relevant pathological feature in ALS and other neurodegenerative disease is the finding of protein aggregates in patient cells, including neurons. A relevant protein involved in ALS is TDP-43 which has been shown to be present in these protein aggregates sequestering functional proteins not only of TDP-43 but also other proteins impiding the execution of their proper function, which would disturb cell function and yield eventually neurodegeneration. [4] The model of lymphoblasts has showed how these cells recapitulated some aspects from the disease such as increased TDP-43 hyperphosphorylation and a TDP-43 mislocalization to the cytoplasm. [5]

Here we have studied protein aggregation using a simple methodology in two different cellular models of ALS, one consisting of the neuroblastoma SH-SY5Y cell line treated with etacrinic acid, and lymphoblasts from both sporadic and genetic ALS patients. We are observing that the proteinopathy aspect of ALS is manifested in both models. By turbidity measurements of protein extracts, a difference in the total amount of protein aggregation comparing sporadic patients with healthy controls is shown. The different mutations of genetic patients lead to differences in the amount of protein aggregation, valuing the importance of measuring individual characteristics. This methodology enables to evaluate promising drug candidates, and we show here how some of them are able to rescue the pathologic aggregation of the patients. The chosen treatments target the inhibition of the phosphorilation of TDP43. Finally the aggregation has also been observed in the model of the SH-SY5Y cell line.

15. Assessment of the therapeutic effect of IGS 2.7, a CK1 protein kinase inhibitor, in combination with riluzol for the treatment of ALS

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Recent evidence in the ALS scientific community suggests this neurodegenerative disease as a TDP-43-pathy. More than 97% of familiar and sporadic ALS patients present TDP-43 aggregates in the affected cells. Our research during the last decade has focused in the recovery of TDP-43 homeostasis with small molecules able to inhibit protein kinases involved in TDP-43 phosphorylation, the main post-translational modification found in TDP-43 aggregates.

The small brain penetrant molecule known as IGS2.7 is a potent and selective protein kinase CK-1 inhibitor that exerts its neuroprotective effect by reducing TDP-43 hyperphosphorylation, reactive gliosis and consequently motor neuron death in a transgenic TDP-43 mouse model. In sporadic ALS immortalized lymphocytes, IGS2.7 recovers TDP-43 homeostasis, decreasing TDP-43 phosphorylation and recovering its functional nuclear localization (1).

In order to translate this promising candidate into the clinic, and considering that riluzol is the standard care for ALS patients, we have evaluated the therapeutic effect of IGS 2.7 in combination with riluzol in lymphoblasts from sporadic ALS patients, whose previous characterization revealed the presence of TDP-43 pathology (2). The combined treatment was found to be synergistic, as we observed higher efficacy in lowering protein aggregation, in decreasing TDP-43 hyperphosphorylation and TDP-43 cytoplasm localization when both drugs were administered simultaneously in lower doses than when they were administered separately. We have also performed an in vivo study of chronic co-treatment using a transgenic TDP-43 model. Preliminary data, also shown a trend in a better behavior when low doses of IGS 2.7 is administered together with riluzol.

Therefore, we propose IGS 2.7 as a promising therapy for the treatment of ALS not only as single treatment but also as an add-on on riluzol standard care.

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2) Posa, D., Martínez-González, L., Bartolomé, F. et al. Recapitulation of Pathological TDP-43 Features in Immortalized Lymphocytes from Sporadic ALS Patients. Mol Neurobiol 56, 2424–2432 (2019).

16. Avoiding TDP-43 cell-to-cell propagation in human sporadic ALS immortalized lymphocytes by treatment with IGS2.7, a CK-1 inhibitor

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Amyotrophic Lateral Sclerosis (ALS) is a devastating progressive neurodegenerative disorder of still unknown etiology that results in loss of motoneurons, muscle paralysis, and death. The main pathological hallmark of ALS, found in 97% of ALS cases, is the presence of TAR DNA binding protein (TDP-43) aggregates in the affected cells.

Human lymphoblastoid cell lines are a useful model to study TDP-43 pathology in ALS and other pathologies as they recapitulate the pathological hallmarks of TDP-43 proteinopathy: fragmentation, cytoplasmic accumulation and hyperphosphorylation [1,2]. Moreover, the prionic behaviour of TDP-43 in Alzheimer's disease lymphoblasts using conditioned medium (CM) experiments has been recently documented [3].

The potential transmission of TDP-43 pathology in immortalized lymphocytes from sporadic ALS patients have been here studied. Results shown a 25KDa TDP-43 fragment in the extracellular medium that may be responsible for disease propagation. A significant increase in TDP-43 pathology such as phosphorylation, fragmentation and cytoplasmic accumulation as well as abnormal cytoskeleton structures in control cells growing in CM of ALS lymphoblasts were detected.

Moreover, we investigated the therapeutic effect of a well-known CK-1 protein kinase inhibitor, the small molecule IGS 2.7, on the ability to prevent the cell- to-cell transmission of TDP-43 pathology. This compound has been previously reported to preserve motoneuron death in an in vivo model of transgenic TDP-43 [4]. Our results show that treatment with IGS2.7 recover functional TDP-43 homeostasis avoiding its prionic propagation, emerging as a good drug candidate for ALS therapy.

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[2] Alquezar C et al. Targeting TDP-43 phosphorylation by Casein Kinase-1δ inhibitors: a novel strategy for the treatment of frontotemporal dementia. Mol Neurodegener. 2016; 11:36.

[3] Cuevas EP et al. TDP-43 Pathology and Prionic Behavior in Human Cellular Models of Alzheimer's Disease Patients. Biomedicines. 2022; 10:385

[4] Martínez-González L et al. Motor neuron preservation and decrease of in vivo TDP-43 phosphorylation by protein CK-1δ kinase inhibitor treatment. Sci Rep. 2020; 10:4449.

17. Axonal mRNA homeostasis in ALS – a link between DNA damage and mitochondria?

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Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive loss of motoneurons. Mutations in the FUS gene have been identified as a cause of ALS leading among other things to accumulation of unresolved DNA damage. Recent studies of motoneurons cultivated in compartmentalized micro fluidic chambers have described defects in mitochondrial transport and membrane potential in the distal parts of axons. Furthermore, these phenotypes could be induced as downstream events after exposure to DNA damaging agents. They exact mechanism of this cross talk, however, remains elusive.

DNA damage can alter the transcriptome and translatome by sequestering of mRNA in stress granules. This could impair axonal mRNA homeostasis by altering the anterograde mRNA transport.

To investigate whether mRNA trafficking deficits exist in ALS, we examined differences in motility and mRNA trafficking under baseline conditions and following DNA-damage induction by UV laser micro irradiation by microscopy of ACTB directed molecular beacons. We found that mRNA trafficking was changed under baseline conditions and responded to UV-laser irradiation induced DNA damage. When we investigated the axonal distribution of the ribosomal protein RPS3 and local axonal protein biosynthesis with a puromycin incorporation assay, we were, however, not able to observe differences between wild-type and FUS-ALS motoneurons. Leading us to the conclusion that the observed mRNA trafficking alterations do not impair protein biosynthesis under the studied conditions.

Further investigations after induction of more severe DNA damage are planned to elucidate whether a dose dependent effect is present.

18. Beneficial effects of CB2 receptor activation at central and peripheral sites in ALS murine models

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The modulation of the endocannabinoid system (ECS), an intercellular communication system which includes receptors, endogenous ligands, and biosynthesis and degradation enzymes, has been proposed as a potential therapeutic option in ALS. Of particular interest is the CB2 receptor (CB2R), which is mainly located in the immune system and other peripheral tissues such as skeletal muscle. In the Central Nervous System (CNS), CB2Rs are mainly located in glial cells at low levels in healthy brain but they become upregulated under pathological conditions. This response has been demonstrated to be protective in ALS, as the pharmacological activation of CB2R preserves motor neurons and reduces glial reactivity in experimental models of ALS. However, less is known about the location (CNS and/or skeletal muscle) of CB2R responsible of these effects, something relevant in ALS which is a neuromuscular disorder. Therefore, our objective in this study was to determine the contribution of neural or muscle CB2R to the neuroprotective effect resulting from their activation in murine models of ALS. To this end, we used two different CB2R agonists developed by Roche Pharma, RO-945, which crosses the blood-brain barrier (BBB) and may act at both neural and muscle compartments, and RO-304, which is peripherally restricted with effects limited to skeletal muscle, in two distinct (SOD1and TDP43) transgenic mouse models. The i.p. administration of both CB2R agonists resulted in a significant improvement in the neurological status and preservation of spinal motor neurons in both transgenic mice, with reduction in microglial reactivity only in the SOD1 transgenic mice. To discard that the beneficial effects of RO-304 may be attributed to BBB damage occurring in experimental ALS models, we analyzed this compound using LC-MS, but RO-304 was not detected in the CNS, confirming that the BBB does not appear to be damaged, that RO-304 was unable to cross the barrier, and that it exerted beneficial effects presumably at the muscle compartment. Therefore, our data challenge the prevailing notion that the activation of CB2R in neural cells is the only reason for the therapeutic benefits of CB2R agonists in ALS, given that these benefits could be seen also with a peripherally restricted agonist. Even, muscle CB2R, which would be activated by both compounds, could be the only responsible of these benefits, but this will require further research.

19. Beta-adrenergic modulation of motoneuronal signaling, electrophysiology and disease-associated transcriptome in the SOD1-ALS mouse model

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Modulation (up- or downregulation) of motoneuron (MN) excitability and synaptic excitation constitutes an important entry point to affect MN degeneration in several MN diseases. We have previously demonstrated that chemogenetic interventions at the level of excitability and of PKA signaling exert profound beneficial effects on synaptic integrity and disease burden in ALS MN. In order to achieve a similar upregulation of PKA signaling and MN firing through natural receptor, we explored the PKA-coupled motoneuronal receptorome in ALS. Among the receptors prioritized by screening available databases (Allen Spinal Cord Atlans, GPCR database) in situ hybridization reveals that adenosinergic, histaminergic, cholinergic and several peptidergic receptors are downregulated, whereas beta-1 adrenergic receptor is distinctively upregulated and the expression of dopaminergic D5 and beta-2 and beta-3 adrenergic receptor are preserved. We explored the acute (3h) and chronic (10days) consequences of beta2/beta3 adrenergic stimulation. In vivo MN electrophysiological recording showed a substantial increase in MN excitability and firing rate upon administration of selective and brain-permeant beta2/beta3 agonists. We used MN microdissection-capture to determine that the increase excitability observed in physiological recordings was mirrored by the upregulation of immediate-early genes. Importantly, acute beta2/beta3 stimulation modified the autophagy-related transcriptional signature, and produced a large-scale change in ion-channel transcription, pointing to impacts on disease pathways and long-term modification in excitability. However, chronic administration of the agonists did not lead to a persistent modulation of excitability, indicating rapid adaptation and desensitization; we detected effects both at physiological and at receptorome level. Our data show that MN display extensive entry points for modulation of their electrophysiological properties, which can be accessed with small molecules with translational potential for ALS treatment.

20. Boosting the peripheral immune response in the skeletal muscles improved motor function in Amyotrophic Lateral Sclerosis (ALS) transgenic mice

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Growing evidence suggests a prominent role of the immune system in ALS pathoprogression, the most common and fatal adult-onset neuromuscular disorder.

We previously reported that MCP1/CCL2, one of the most potent pro-inflammatory chemokines, is strongly upregulated in the nervous system of C57 slow-progressing than 129Sv fast-progressing SOD1G93A mice, the latter showing a poor immune response and an accelerated peripheral nerve and muscle degeneration.

Given the pivotal role of MCP1-mediated signalling in driving damaged axons and muscle regeneration, we boosted the chemokine along the motor unit of the two SOD1G93A models through a single intramuscular injection of a self-complementary adeno-associated virus serotype 9 vector engineered with the Mcp1 gene (scAAV9_MCP1).

Our observations revealed that the scAAV9_MCP1 injection had an opposite effect on the clinical phenotype of the two ALS models. C57SOD1G93A mice responded positively to MCP1 boosting, anticipating leucocyte recruitment and phenotypic switch from the pro- to the anti-inflammatory fingerprint within the peripheral compartment. This sustained the activation of the myogenic programme and nerve regeneration, finally slackening off the motor symptoms progression. Conversely, 129SvSOD1G93A mice exhibited a delayed activation of the pro-inflammatory immune muscle response upon MCP1 boosting, exacerbating the toxic inflammation and eventually worsening the motor ability late in the disease.

We provided direct evidence underlying the pivotal role of the immune response in driving skeletal muscle regeneration, spotlighting its nature and temporal activation as limiting factors to preserve the periphery and interfere with the ALS course tangibly.

Intriguingly, our data showed a novel immune-unrelated role of MCP1 in promoting motor axon regeneration and modulating neuroinflammation in the nervous system of SOD1G93A mice, with the overall effect of reducing neurodegeneration.

Altogether, these observations highlight the immune response as a critical determinant for disease variability and proffer a reasonable explanation for the failure of systemic immunomodulatory treatments suggesting new potential strategies to hamper ALS progression.

21. C9ORF72 deficiency results in degeneration of the vertebrate retina in vivo

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Hexanucleotide repeat expansions within the gene C9ORF72 are the most common cause of the neurodegenerative disease spectrum FTD/ALS and lead to a reduction in C9ORF72 expression levels. Therefore, to understand the consequences of C9ORF72 deficiency in vivo we characterised an aged c9orf72 loss of function zebrafish line. Analysis of the spinal cord showed an absence of pathology, however, detailed examination of the retina showed prominent features of neurodegeneration within c9orf72-/-. GFAP expression within Muller glia was redistributed to be more pronounced within the apical end of the cell, suggesting gliosis in the aged mutant retina. Microglia, although unchanged in number, where abnormally distributed in c9or72-/- to the photoreceptor layer. Furthermore, there was marked thinning of the retina and a reduction in different neuronal subtypes in c9or72-/-. To our knowledge this is the first reported instance of spontaneous neurodegeneration in the context of C9ORF72 deficiency in vivo.

22. C9orf72-ALS iPSC microglia are pro-inflammatory and induce apoptosis of co-cultured wild-type motor neurons via MMP9

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Background

A growing body of evidence supports a role for neuroinflammation in ALS pathophysiology, with microglia strongly implicated. Particularly in C9orf72-ALS patients, widespread microglial activation is observed and correlates with disease progression. In neurons, the hexanucleotide repeat expansion (HRE) mutation in C9orf72 has been shown to result in toxicity through both loss-of-function and gain-of-function mechanisms. Investigating C9orf72 loss-of-function in mice, C9orf72 knock-out led to a pro-inflammatory state in myeloid cells and microglia. Here, we used human induced pluripotent stem cell (iPSC)-derived microglia to study the consequences of the C9orf72 HRE on human microglia.

Methods

We differentiated iPSC-derived microglia from three C9orf72-ALS patients, three healthy controls, and one isogenic line. We analysed C9orf72 HRE mutant microglia in monoculture and co-culture with iPSC-derived wild-type motor neurons, with and without LPS treatment ('M0'/'M1' phenotype).

Results

iPSC-derived microglia displayed typical microglial morphology and expressed key microglial markers. C9orf72 protein expression was significantly reduced in C9orf72 HRE mutant microglia compared with healthy controls, indicating C9orf72 loss-of-function. In addition, the DPRs Poly(GA)/(GP) were detectable in C9orf72 HRE mutant microglia, demonstrating the presence of gain-of-function products. Transcriptomic analysis revealed enrichment of pathways associated with immune cell activation, cytokines, lysosomes, and the extracellular matrix in C9orf72 HRE mutant microglia, particularly after LPS priming. Specifically, we identified consistently increased expression and release of matrix metalloproteinase-9 (MMP9) in LPS-primed C9orf72 HRE mutant microglia compared with both healthy and isogenic control microglia. LPS-primed, but not unstimulated, C9orf72 HRE mutant microglia induced apoptosis in co-cultured wild-type motor neurons, which was ameliorated by concomitant application of an MMP9 inhibitor.

Conclusions

These results demonstrate cellular dysfunction of C9orf72 HRE mutant microglia, and a non-cell-autonomous role in driving C9orf72-ALS pathophysiology in motor neurons through MMP9 signalling.

23. Cellular and axonal transport phenotypes due to the C9orf72 HRE in iPSC-motor and sensory neurons

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Introduction: Induced pluripotent stem cell (iPSC)-derived motor neurons (MNs) from patients with Amyotrophic Lateral Sclerosis (ALS) who are carriers of the C9orf72 hexanucleotide repeat expansion (HRE) have multiple cellular phenotypes, but it remains unknown whether these phenotypes contribute to the cell-specific vulnerability seen in this disease. We took advantage of the capability of iPSCs to generate different cell types, to compare existing MN phenotypes due to the C9orf72 HRE with sensory neurons (SNs), which are relatively spared in ALS.

Methods: We differentiated iPSC-MNs and SNs from five controls and three patients carrying the C9orf72 HRE and performed an in-depth comparison of the obtained cell types using immunofluorescence, western blotting, and RNA sequencing analysis. C9orf72 phenotypes were assessed using dipeptide ELISA and fluorescence in situ hybridisation. Survival, stress granule formation and TDP-43 mislocalisation were assessed with and without 0.5 mM sodium arsenite treatment for one hour. Axonal transport was studied using microfluidic devices using Lysotracker, Mitotracker and Cholera Toxin.

Results: RNA sequencing confirmed the divergent transcriptome of iPSC-MNs and SNs and expression of their relevant cell type markers. There was a significant but weak overlap between the iPSC model and healthy adult single cell and bulk postmortem datasets. Sense and antisense RNA foci and dipeptide protein synthesis were confirmed in both MNs and SNs from C9orf72 patients. Compared to SNs, MNs neurons had fewer arsenite-induced stress granules ($p<0.01$) and a higher relative cytoplasmic concentration of TDP-43 ($p<0.001$), but no differences were observed due to the C9orf72 HRE. The speed of retrograde lysosomal and bidirectional mitochondrial axonal transport was reduced in both C9orf72 MNs and SNs compared to controls ($p<0.05$).

Conclusion: iPSC-MNs and SNs carrying the C9orf72 HRE have similar levels of RNA foci and dipeptide protein, and both display the same axonal transport deficits, indicating that the studied phenotypes are not likely responsible for the cell type selectivity of ALS in isolation. The detailed molecular and transcriptomic comparisons of MNs and SNs carried out in this study confirm the utility of the iPSC neuronal models for studying selective vulnerability, but also emphasize the marked differences to their corresponding adult cell types, and provide a valuable resource for future work in this area.

24. Cellular Stress induces Neuron-specific Degradation of the Fragile X Protein Family

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The fragile X protein (FXP) family comprising the multifunctional RNA-binding proteins FMR1, FXR1 and FXR2 has recently been implicated in several neurodegenerative diseases. Here, independent lines of evidence point towards loss of function and/or expression of these proteins early in the disease, or even at the pre-symptomatic stage. In Alzheimer's and Parkinson's disease, decreased expression of FMR1 has been linked to the formation of amyloid plaques and Lewy bodies, respectively. In amyotrophic lateral sclerosis (ALS), overexpression of FMR1 substantially mitigated the phenotype of FUS- and TDP-43-linked in vivo models, and aberrant expression of the FXPs was evident in human post mortem spinal cord tissue independent of the underlying cause of the disease. Here, especially loss of FXR2 correlated with the occurrence of FUS aggregates. Therefore, molecular mechanisms regulating expression of the FXPs are of high interest, and may represent starting points for novel therapeutic approaches. In this study, we found that cellular stress induces rapid decline of FMR1 and FXR2 protein while the effect on FXR1 was much less pronounced. Specifically, ER and osmotic stress, but not oxidative stress or induction of DNA damage, led to decreased level of the FXPs. This decline was specific for neurons, and evident in mouse primary cortical neurons and human H4 neuroglioma cell line, but not in HEK293 cells. Interestingly, the decrease of the FXPs was independent of stress granule formation and not reflected at the mRNA level. Additionally, decreased FXP expression was too fast to be explained by decreased translation. We therefore conclude that specific cellular stress induces the rapid degradation of the FXPs. Molecular mechanisms involved, as well as relevance of these findings for human ALS are currently under investigation.

25. Characterising novel, humanised and physiological mouse models of FUS-ALS

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Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative condition, with mutations in the FUS gene accounting for particularly severe, early onset forms of the disease. Despite extensive research into ALS, there is still no cure, and little in the way of efficacious treatments. This is in part due to a lack of physiologically relevant models. Mouse models of ALS, and other neurodegenerative diseases, have historically been transgenic and as such, the phenotypes they display may represent artefacts of overexpression, rather than disease relevant mechanisms. Here, I will present data on a humanised knock-in mouse model of FUS-ALS, whereby the mouse Fus gene from the ATG start codon through to the 3'UTR, including all introns and exons, has been replaced with the human FUS sequence at the endogenous Fus locus. An ALS patient mutation, P525L, was then introduced into the FUS gene using CRISPR/Cas9. This humanised ALS model expresses the mutant FUS protein at physiologically relevant levels, and in a normal pattern of expression. Heterozygous humanised FUSP525L mice show disease relevant phenotypes, including progressive late-onset reduction in muscle strength from 1 year, hyperactivity and metabolic impairment. This is alongside cellular and molecular changes from 4 months, as shown by RNA sequencing data from the spinal cord and tibialis anterior muscle, and primary motor neurons. The phenotypes displayed by this model highlight the potential of fully humanised knock-in mice to aid in unravelling early disease mechanisms, and ultimately produce therapies targeted towards the human gene and protein, in the context of, but not limited to, ALS.

26. Characterising pathological oligomers with single-molecule microscopy

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More than 95% of ALS cases demonstrate mislocalised and aggregated TDP-43 protein accumulation in the central nervous system at post-mortem examination. However, despite this consistent pathomolecular aetiology, the clinical manifestations and disease progression can vary widely. To unpick this complexity, detailed characterisation of pathomolecular heterogeneity is much needed, in particular in the earliest oligomeric species that are increasingly believed to drive neurotoxicity. However, such studies are hindered by the difficulty of profiling nanoscale oligomeric species. To address this, we have created the ConTOR (constituent topography at oligomer resolution) imaging platform, custom-designed to characterise pathological oligomers at unprecedented detail, and in sufficient numbers, to quantify molecular signatures of disease state. ConTOR combines surface-immobilisation of single aggregates with single-molecule imaging capable of resolving multiple targets down to 20 nm resolution in a fully automated set up.

Using α-synuclein as a model of proteinopathy to establish proof-of-concept data, ConTOR has enabled the observation of differences in the aggregate surface profile between recombinant protein aggregates used to model disease in vitro and the pathological aggregates derived from human tissue. Adaptations and antibody panels are now being developed to investigate the composition, size, abundance, stability, and cell preponderance of TDP-43 aggregates in ALS patient tissue. Through correlation with clinical phenotypes, we aim to identify high-risk aggregate profiles as novel biomarkers of disease. This work is funded by a Lady Edith Wolfson Fellowship with the Motor Neurone Disease Association.

27. Characterization of a therapeutic approach to target TDP-43 proteinopathy using phage display-derived scfv

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A major hallmark of amyotrophic lateral sclerosis (ALS) is the presence of toxic cytoplasmic aggregates of the TAR DNA/RNA binding protein (TDP-43) in the motor neurons and glial cells of 97% of patients, making this protein an important link between familial and sporadic ALS, and a major therapeutic target. The aim of our study is to develop biotherapeutics targeting different aspects of TDP-43 proteinopathy.

By means of phage display, we identified single chain variable fragment (scFv) clones against wildtype TDP-43. In silico binding prediction revealed the potential binding sites of the scFv's including the N- and C-termini and RRM1/2 of TDP-43. Their interaction with TDP-43 was confirmed using ELISA and Surface Plasmon Resonance (SPR; KD=3.1E-9). Immunofluorescence and MTT reduction assays demonstrated the non-cytotoxicity and robust expression of the scFv's, respectively. We tested the effect of the scFv on TDP-43 proteinopathy in HEK293T and NSC-34 cells overexpressing wildtype TDP-43. The scFv's colocalized with cytoplasmic and aggregated TDP-43. One scFv (D7) decreased the level of the insoluble 35 kDa C-terminal fragment of TDP-43. This decrease seems to be mediated by the proteasome. Another scFv (B1) decreased the activation of NF-- B caused by TDP-43 overexpression. Both scFv's also seemed to reverse some TDP-43-induced metabolic alterations, particularly linked to the lipid metabolism.

To enhance the efficiency and specificity of the scFv's delivery to target cells, we complexed them to PEGylated superparamagnetic iron oxide nanoparticles (SPIONs). Different mass ratios of SPION to scFv were tested for their size, zeta potential, and scFv retention capacity. HEK293T cells were treated with the selected PEG-SPION-scFv formulations to test their internalization. Flow cytometry and western blot analyses confirmed the cellular internalization of the SPIONs and scFv's, respectively. Interestingly, there was a 30-fold increase in the internalization of the scFv when complexed to SPIONs compared to a commercial protein delivery reagent.

In conclusion, we have successfully developed two scFv's specific to wildtype TDP-43 and able to counteract different aspects of TDP-43 pathology. To our knowledge, this is the first time that scFv's are complexed to SPIONs for targeted delivery. Further studies will assess the toxicity of PEG-SPION-scFv and their effect on TDP-43 pathology in both in vitro and in vivo ALS models.

28. Characterization of TDP-43 role in cellular models exposed to ALS-related stress

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Dysfunction of transactive response DNA-binding protein of 43 kDa (TDP-43) is a common hallmark of Amyotrophic Lateral Sclerosis (ALS), a devastating disease with no known cure. TDP-43 dysfunction is linked to the impairment of RNA metabolism, energy regulation, and oxidative stress present in ALS. We generated a human cell line (HeLa) with an inducible silencing system to knock down TDP-43 expression, to model the effects of TDP-43 loss on cellular homeostasis traits, including metabolic activity, oxygen consumption, and cell death. We also exposed the model to energetic and ferroptosis stressors. Our results indicate that TDP-43 function is crucial for maintaining cellular homeostasis, significantly impacting cell metabolism, oxygen consumption, and viability. Notably, TDP-43 loss impaired cellular growth in association with mitochondrial inefficiency. These findings provide evidence that metabolic activity and oxygen consumption are intimately linked to TDP-43 function, underscoring the importance of this protein in ALS pathogenesis. Overall, our cellular model provides new insights into the complex pathophysiology of ALS highlighting TDP-43 critical role in cellular homeostasis. These findings could pave the way for new therapeutic strategies targeting TDP-43 in ALS and other neurodegenerative diseases.

29. Characterization of the KIF5A aggregates in patient-derived induced pluripotent stem cells

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Our group has previously described heterozygous ALS-causing mutations in the Kinesin Family Member 5A (KIF5A). These mutations are located in the C-terminal tail and have been predicted to affect the splicing of exon 27, altering the globular cargo-binding domain of the kinesin. Indeed, a mutation resulting in KIF5A exon 27 skipping (ΔExon27) has been shown to cause altered protein and RNA interactions. Moreover, we recently found that the conformational changes caused by ΔExon27 is inducing neurotoxicity by abolishing KIF5A autoinhibition. We obtained hiPSCs from one pre-manifest carrier of a heterozygous ΔExon27 mutation (c.3020+2T>C) and from two ALS patients of a family with a c.2993-1G>A heterozygous mutation, and differentiated them into motor neurons. We showed that these mutations alter exon 27 splicing differently, yet they both lead to the production of a common C-terminal aberrant 39 amino acid end. In addition, patient-derived motor neurons displayed a significant increase in KIF5A inclusions in comparison to control, in accordance with recent publications showing that ΔExon27 is prone to form cytoplasmic aggregates when overexpressed in different cell lines. Here, we characterized these aggregates in three control and three mutation-harboring induced motor neuron cell lines at different time points based on their number, size, intensity and subcellular localization to serve as the starting point for the development of a pre-clinical platform to expand the mechanistic understanding of KIF5A/ALS pathology, and the development of new pharmaceutical strategies.

30. CLIP-Seq Analysis Enables the Design of Ribosomal RNA Bait Oligonucleotides That Protect Against C9ORF72 ALS/FTD-Associated Poly-GR Pathophysiology

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Amyotrophic lateral sclerosis and frontotemporal dementia patients with a hexanucleotide repeat expansion in C9ORF72 (C9-HRE) accumulate poly-GR and poly-PR aggregates. The pathogenicity of these arginine-rich dipeptide repeats (R-DPRs) is thought to be driven by their propensity to bind to low complexity domains of multivalent proteins. However, the ability of R-DPRs to bind native RNA and the significance of this interaction remains unclear. We used computational and experimental approaches to characterize the physicochemical properties of R-DPRs and their interaction with RNA. We find that poly-GR predominantly binds ribosomal RNA (rRNA) in cells and exhibits an interaction that is predicted to be energetically stronger than that for associated ribosomal proteins. Critically, modified rRNA "bait" oligonucleotides restore poly-GR-associated ribosomal deficits in cells and ameliorate poly-GR toxicity in patient neurons and Drosophila models. Our work strengthens the hypothesis that ribosomal function is impaired by R-DPRs, highlights a role for direct rRNA binding in mediating ribosomal disfunction, and presents a strategy for protecting against C9-HRE pathophysiological mechanisms.

31. Combined treatment with nicotinamide riboside, pterostilbene and ibudilast delays motor neuron death in murine models of ALS

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Background: Oxidative stress and neuroinflammation are major mechanisms in the pathophysiology of amyotrophic lateral sclerosis (ALS). Combination of nicotinamide riboside (NR, a NAD+ promoter) and pterostilbene (PT, a natural antioxidant) delays progression in ALS patients and in SOD1G93A transgenic mice. Ibudilast (IBU) targets different phosphodiesterases and has shown neuroprotective properties in models of ALS through different mechanisms: decrease of proinflammatory cytokines, macrophage migration inhibitory factor and glial cell activation. Early-phase studies suggest that IBU may improve survival outcomes and slow disease progression in ALS patients. Thus, we investigated if IBU can enhance the effects of NR and PT and slow down the progression of ALS in murine models.

Methods: The effectiveness of NR, PT, and/or IBU treatment on the progression and survival of SOD1G93A and FUS-R521C mice was evaluated. Assessment of neuromotor activity and coordination was correlated with histopathologic changes, and measurement of proinflammatory cytokines in the cerebrospinal fluid (CSF). nOxidative/nitrosative stress, cell death, and mitophagy were studied in motor neurons (MNs) isolated from the ALS mice.

Results: IBU improved the effect on survival and neuromotor functions elicited by NR and PT. The triple combination decreased the microgliosis and astrogliosis associated to ALS progression, and the levels of proinflammatory cytokines in the CSF. TNFa, IFNg and IL1b increased H2O2 and NO generation by MNs, astrocytes, microglia and endothelial cells isolated from ALS mice. NR and PT decreased H2O2 and NO generation in all these cells. IBU was efficacious in decreasing levels of TNFa in the CSF and the generation of H2O2 by microglia and endothelial cells. Exposure of MNs to pathophysiological concentrations of H2O2 or NO caused minimal cytotoxicity. However, H2O2-induced MN cytotoxicity was highly increased by NO due to the formation of potent oxidants, presumably .OH and -OONO radicals, via a trace metal-dependent process.

Conclusion: The combined treatment (IBU+NR+PT)delays ALS progression and increases survival in mice models. An increase of antioxidant defenses in the MNs and a drastic decrease in microgliosis, astrogliosis and the levels of proinflammatory cytokines in the CSF are linked to the underlying mechanisms. The survival benefit of this novel three-agent treatment is not achieved with either a single agent dual combinations.

32. Contribution of inhibitory neurons to ALS and FTD-like phenotypes linked to FUS

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Multiple studies suggest that inhibitory neurons are involved in ALS. Indeed, patients show impaired intracortical inhibition prior to motor symptoms onset, and post-mortem studies highlight molecular alterations of cortical and spinal inhibitory circuits. Recently, we identified inhibitory defects in an ALS-FTD mouse model based on the expression of mutant Fused in Sarcoma (FUS).

Mutations in FUS cause severe forms of ALS, particularly when its nuclear localisation signal (NLS) is truncated. This induces the cytoplasmic mislocalisation of FUS, which is also observed in ALS and FTD patients devoid of mutations. In heterozygous mice, the constitutive NLS deletion, and subsequent cytoplasmic delocalisation of FUS, led to cortical hyperactivity associated with molecular and ultrastructural alterations of GABAergic synapses, ALS-like motor impairments and FTD-like behavioural dysfunctions.

To characterise the contribution of inhibitory neurons to these phenotypes, we created a mouse model based on a constitutive NLS deletion selectively in GABAergic neurons. Interestingly, when both copies of Fus were mutated, we observed a progressive alteration of the post-natal body development, as evidenced by reduced weight and limb strength. Furthermore, half of the homozygous pups did not survive weaning. When only one copy of Fus was mutated, mice were able to reach adulthood but males displayed FTD-like social abnormalities. In order to pinpoint the underlying mechanisms, we performed in vivo two-photon calcium imaging, where we observed an increase in neuronal activity levels in the frontal cortex of anaesthetised animals. Our results suggest that FUS mislocalisation in inhibitory neurons only is sufficient to cause cortical hyperexcitability and FTD-like impairments. In a complementary strategy, we created a mouse model expressing Fus truncation in every cell type except inhibitory neurons. This genetic rescue approach proved to be sufficient to delay ALS-like motor defects in females. Ultimately, with this study, we will determine if Fus mutations in inhibitory neurons are sufficient and/or necessary to induce ALS and FTD-like symptoms.

33. Contribution of one-carbon metabolism to neurodevelopment and neurodegeneration in mouse models of ALS

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With a few exceptions, the vast majority of neurodegenerative diseases (NDD) manifests during adulthood, implicitly suggesting that these diseases only hit the adult and fully mature central nervous system. However, NDD onset follows a long prodromal phase, shifting the origins of the disease to younger ages. In addition, genetic cases exist that display juvenile onset. Together, these have contributed to the emergence of possible neurodevelopmental roots of at least subsets of NDD cases. While seemingly different, neurodevelopmental and NDD share common mechanisms. Amongst those are the alterations of the one-carbon metabolism (1C), which combines the folate and methionine cycles respectively involved in purine synthesis and methylation reactions, and relies on four key enzymes: DHFR, MTHFR, AHCY and MAT2A.

My Ph.D. project aims at investigating whether ALS might arise from neurodevelopmental impairments, focusing on the disease-relevant population of corticospinal neurons (CSN, aka upper motor neurons), and on 1C metabolism. We formerly demonstrated that developmental absence of CSN delays disease onset and extends survival in the Sod-1G86R mouse model of ALS. We hence hypothesize that alterations in 1C metabolism could impact the development of CSN and contribute to ALS onset later in life. To test this hypothesis, 1C metabolism will be characterized and manipulated in the developing CSN of mouse models of ALS (Sod1G86R & FusΔNLS) using epigenetics, transcriptomics, metabolomics, genetics and pharmacology. Using RNAscope and RNAseq, I have started characterizing the pattern of expression of the key regulators of 1C during CSN development in ALS mice. My preliminary data indicates that Mthfr expression picks when CSN become post-mitotic and is maintained later on at a lower level. Importantly, its expression is downregulated in the CSN of Sod1G86R mice compared to controls. I have also identified a putative polymorphism of AHCY in ALS, and an upregulation of its expression in the CSN of Sod1G86R mice compared to controls. Work is ongoing to understand when alterations of 1C occurs in ALS mouse models, and what are the consequences on brain development, disease onset and progression.

Because 1C metabolism also strongly relies on diet, my project may not only inform on the consequences of ALS-related mutations on brain development, but also on developmental environmental/dietary restrictions on the motor system and potentially the onset of sporadic ALS.

34. C-terminal frameshift variant of TDP-43 with pronounced aggregation-propensity causes rimmed vacuole myopathy but not ALS/FTD

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Neuronal TDP-43-positive inclusions are a neuropathological hallmark in frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), accounting for 45% and 97% of cases, respectively. Missense variants in TARDBP, the gene encoding for TDP-43, can be solely causal for ALS and predominantly cluster in the C-terminal region. This region of the protein harbours a prion-like domain (PrLD), responsible for modulating the liquid-liquid phase separation and aggregation properties of the protein. TDP-43 accumulations have also been described in rimmed vacuole myopathies, including sporadic inclusion body myositis. Nonetheless, myopathy-causing TDP-43 variants have not yet been reported. In this work we conclusively identified a frameshift mutation in the TARDBP gene, causing the production of a C-terminally altered TDP-43 variant (TDP-43 p.Trp385IlefsTer10), segregating in a five-generation Belgian family using whole exome sequencing and genome-wide linkage analysis (maximum multipoint LOD-score of 3.61). Patient-derived muscle biopsies showed clear signs of a myopathy with rimmed vacuoles with the identification of TDP-43-positive sarcoplasmic accumulations and an increased number of autophagosomes. Liquid-liquid phase separation assays revealed that TDP-43 p.Trp385IlefsTer10 does not form droplet-like condensates, instead quickly forming fibril-like bodies, suggesting increased aggregation propensity compared to wild-type TDP-43 and ALS-causing TDP-43 variants. Transcriptomic analysis of patient muscle uncovered a number of abnormally spliced sarcomeric genes, with TTN and NEB among the genes with the highest number of events, and a signature suggestive of increased muscle regeneration. In Drosophila models, TDP-43 p.Trp385IlefsTer10 behaved as a partial loss-of-function allele as it was able to rescue the TBPH (fly ortholog of TARDBP) neurodevelopmental lethal null phenotype while showing strongly reduced toxic gain-of-function properties upon overexpression. This loss of toxic gain-of-function properties were also confirmed in primary rat neurons. Altogether, these genetic, pathological, in vitro and in vivo results demonstrate that TDP-43 p.Trp385IlefsTer10 is an aggregation-prone TDP-43 variant causing an autosomal dominant vacuolar myopathy, but not ALS/FTD, suggesting a novel tissue-specific mechanism of disease for TDP-43 in muscle and genetically linking TDP-43 proteinopathy to myo-degeneration and expanding the range of TDP-43 proteinopathies.

35. Cyclodextrins As Therapeutic Agents in a poly-GA Mouse Model

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Amyotrophic lateral sclerosis (ALS) is a rapidly fatal neurodegenerative disease due to progressive loss of upper and lower motor neurons with no disease modifying therapy. A hexanucleotide expansion in C9orf72 is the most common known cause of ALS found in ~40% of familial and ~5% of apparently sporadic patients. All forms of ALS are characterized by massive neuroinflammation, particularly in the corticospinal tract, and a sharp increase of inflammatory biomarkers at disease onset. Originally, this project aimed to address the role of neuroinflammation in disease progression in an aggressive poly-GA (a C9orf72 DPR) ALS model using a CSF1R inhibitor which has a dampening effect on microglial response without depleting microglia. However, we came across a serendipitous discovery where 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD), the vehicle for our CSF1R inhibitor, was presenting a significant improvement in the lifespan of the poly-GA mice. Further investigations including single-nuclei RNAseq (snRNAseq) with over 230000 nuclei revealed that cholesterol metabolism is severely affected in our model, where biosynthesis of cholesterol is downregulated while export is upregulated. The beta-cyclodextrin in our vehicle has had a rescuing effect predominantly on oligodendrocytes, where Serpina3n, a marker of disease associated oligodendrocytes (DOLs) that is upregaulted due to disease, is partially rescued upon treatment. The same effect was also observed with various other disease-associated genes in oligodendrocytes. We have not observed an appreciable change in NfL levels at end-stage, which is at a later timepoints due to the extended survival, implying that modulating oligodendrocytes is sufficient for employing a meaningful therapeutic approach in our model. A second treatment cohort confirmed our findings, and further showed that Hydroxypropyl-γ-cyclodextrin (HP-γ-CD) is even more effective to extend the lifespan. Currently we are validating our sequencing results and performing lipidomics to get a clearer view of the picture. The blood brain barrier in our model is likely compromised, which could enable cyclodextrin to get into the brain and exerts its effect. Therefore, more models should undergo treatment prior to clinical applications.

36. CYP46A1 as a relevant target to treat ALS pathology independant from its origin

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Background: Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease and is characterized by the progressive loss of upper and lower motor neurons, leading to paralysis and death. Accumulation of cholesterol in the central nervous system (CNS) has been reported to actively contribute to the disease progression in Alzheimer's disease, Huntington's disease, Spinocerebellar ataxia and more recently ALS. Cholesterol is essential or myelin compartment, but also for its functional and structural role in plasmatic membrane. However, in the CNS, cholesterol is synthetized in situ and is not able to freely cross the blood brain barrier (BBB). Cholesterol-24-hydroxylase (CYP46A1) allows the conversion of cholesterol to 24- hydroxycholesterol, able to cross the BBB, thus regulating cholesterol homeostasis. Furthermore, this enzyme is a key neuronal stress response such as oxidative stress or protein aggregation.

Objective: Therefore, we hypothesized that CYP46A1 could be relevant for a therapy in ALS to target both familial and sporadic forms of ALS independently from their genetic origin.

Methods: We used 2 mouse models of ALS: the severe SOD1G93A model and C9ORF72 model with expansion, that we treated using AAV intravenous delivery at pre or post symptomatic stage.

Results As a first step, we confirmed that the AAV has a specific tropism for the CNS and especially motoneurons. Secondary, we demonstrated a significant and prolonged motor rescue of animals treated pre or post-symptomatically, but also a preventive effect on motoneuron degenreataion, myelin loss, compared to untreated animals but also a significant rescue of muscle and neuromuscular junction phenotype as well as a complete rescue of misfolded SOD1 aggregation. Moreover, out therapy is also efficient in another model of the ALS: the C9ORF72 expansion model with a prevention/correction of the behavior abnormalities.

Conclusion: CYP46A1 is a relevant target for ALS treatment independent from its origin. In addition, we developed new strategies for delivery in large animals with non invasive approach.

37. Cytokines dysregulation in spinal cord organoids derived from sALS patients

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Amyotrophic lateral sclerosis (ALS) is a non-cell autonomous disorder as many cell types contribute to motor neurons death. The lack of effective treatments is probably due to the absence of a realistic model that can recapitulate pathogenic mechanisms. Cerebral organoids are pluripotent stem cell-derived self-organizing structures that allow in vitro generation of the tissues. We developed a new method for the generation of spinal cord organoids (SCOs) that can be used for the study of pathogenic mechanisms in ALS. Aim of the work was to characterize a 3D organoid model for the study of ALS pathogenesis. We started from iPSCs obtained from healthy controls and sporadic ALS (sALS) patients. We differentiated iPSCs into neural stem cells (NSCs). We dissociated NCSs using StemPro Accutase and a cell strainer. Then, we plated NSCs on low-attachment plates and we cultured them in floating conditions using an orbital shaker. We differentiated NSCs to generate SCOs. We then characterized cells by phase-contrast and confocal microscopy. We found that SCOs derived from sALS patients were smaller and with irregular morphology compared to healthy controls. Using the GFAP marker, we found that sALS organoids have a thicker glial layer compared to healthy controls. We also found that healthy controls organoids show longer neurites compared to sALS organoids. Finally, we found a diverse composition of cell populations. Indeed, healthy controls organoids show a higher amount of differentiated cells compared to sALS organoids. We investigated cytokines released in culture supernatant of SCOs, and we found several differences between ALS patients and healthy controls organoids. In particular, we reported the upregulation of ApoA1, CD30, EGF, GM-CSF, MMP-9, OPN, ADSF/RETN and the downregulation of Ang-1, HGF, IGFBP3, IL8, MCP-1, VCAM1. In conclusion, our data suggest that SCOs represent a promising tool for the investigation of pathogenic mechanisms of ALS, such as cytokines dysregulation.

38. Deciphering the role of Tbk1 in mouse motor neurons and microglial cells, and its implications for ALS pathogenesis.

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Mutations in the ubiquitously expressed TANK-Binding Kinase 1 gene (TBK1) are linked to Amyotrophic Lateral Sclerosis (ALS) and act by a dominant loss-of-function mechanism. TBK1 is involved in autophagy and innate immunity, suggesting that TBK1 mutations could lead to ALS by both cell-autonomous (autophagy deregulation in motor neurons) and non cell- autonomous mechanisms (altered responses in microglia).

To decipher the role of TBK1 in these two cell types, we have generated mice with Tbk1 deletion specifically in spinal cord motor neurons or microglia using a cre/lox approach. Both mouse models have normal lifespans, but, surprisingly, using aging as an additional stressor, we found that Tbk1 deletion induces much more transcriptional changes in aged microglia than aged motor neurons. In motor neurons, loss of Tbk1 nevertheless leads to a persistent presence of p62+ inclusions throughout life and increased age-related neuromuscular junction alterations, but does not induce loss of motor neurons.

To study the role of Tbk1 in microglia, we first cultured primary mouse microglial cells and showed that Tbk1 deletion decreases their responses to pro-inflammatory stimuli LPS and polyI:C, suggesting a beneficial effect under neuroinflammatory conditions. In parallel, we studied microglia in vivo, isolated by FACS from brains of young and aged mice, and found major transcriptional effects of Tbk1 deletion on microglial cells, already present from young age, but also with additional changes during aging. Using transcriptomics and histology, we were able to put in perspective the phenotype of Tbk1-deleted microglial cells with the changes happening during normal microglial aging.

In order to investigate whether the changed phenotype of microglia with Tbk1-deletion has a beneficial or deleterious impact in the context of motor neuron stress, we are currently studying this in two complementary models of acute and chronic motor neuron injury. Since patients with TBK1 mutations can also suffer from FTD in addition to ALS, and considering the possibility that microglial deregulations can affect synaptic integrity, we are also assessing if Tbk1 deletion in microglia could produce FTD-like social behavior defects in mice.

This project could help better understand TBK1-mediated ALS pathogenesis, and more generally the contribution of pathological microglial deregulations to neurodegeneration.

39. Defects in the DNA damage response are Rescued by Enhancing Chromatin Ubiquitination and DDRNAs Biogenesis in ALS Cellular Model System.

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DNA double strand breaks (DSBs) are cytotoxic lesions which activate the DNA Damage Response (DDR) cascade, initiated by histone H2A.X phosphorylation (γH2A.X) and RNF168 E3 ligase-dependent ubiquitination (H2AK13/15Ub). Along with these chromatin modifications, damage-induced long non-coding RNAs (dilncRNAs) and the short DROSHA-dependant DNA damage RNAs (DDRNAs) are necessary for downstream DDR activation and 53BP1 recruitment, which ultimately channels repair towards non-homologous end joining (NHEJ) in non-cycling cells. Notably, post-mortem neurons from Amyotrophic Lateral Sclerosis (ALS) patients carrying FUSP525L cytoplasmic inclusions (CI) exhibit an aberrant DNA damage accumulation. Elucidating the DDR defects underlying the DNA damage accumulation in ALS patients can be pivotal for uncovering new therapeutic strategies. To this end, we express FUSP525L in Hela cells to recapitulate the pathological formation of CI. Indeed, we found persistent DNA damage accumulation, detected as high pan-nuclear γH2A.X signal, coupled with widespread chromatin structure alterations, indicated by a distribution shift of heterochromatin markers in FUSP525L CI cells. These changes correlated with a nuclear-wide damage-induced transcriptional repression, reminiscent of a defect in NHEJ. Consistently, we found that FUSP525L CI formation hindered RNF168 recruitment to damaged chromatin, subsequently impeding histones ubiquitination and 53BP1 foci formation. The USP51 de-ubiquitinating (DUB) enzyme was demonstrated to counteract RNF168 signaling by removing ubiquitin from the H2AK13/15 site. We hypothesized that USP51 inactivation could be beneficial to restore 53BP1 foci and NHEJ. Interestingly, we observed that depletion of USP51 in cells bearing FUSP525L CI increased chromatin ubiquitination, and rescued 53BP1 foci formation while reducing γH2A.X signal and ameliorating transcriptional repression, suggesting the restoration of a proficient DNA repair. Importantly, these effects were dependent on USP51 catalytic activity and prompt the possibility to pharmacologically inhibit this DUB to promote a chromatinic context that favors DNA repair in FUSP525L CI cells. In parallel, Enoxacin administration, a compound that enhances DDRNAs production and DSB repair, was also efficient to ameliorate the observed DDR defects, which suggests a possible usage of this drug as a second therapeutic strategy to improve DNA damage response and counteract ALS-related neuronal death.

40. Defining the role of histone H1.2 in the pathogenesis of FUS associated Amyotrophic Lateral Sclerosis

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The cause underlying ALS is not known in 90% of cases, while only 10% present familial history. Over 30 genes are associated with the disease, majority of which codes for RNA-binding proteins (RBPs). Remarkably, distinct ALS-related RPBs with low-complexity domains can accumulate into membrane-less ribonucleoprotein organelles that form when the cell is under stress, known as stress granules (SGs). Here we focus on studying FUS protein, an RBP linked with the great majority of severe juvenile ALS cases. We observe that FUS mutations lead to more SG formation and delayed recovery dynamics. To study the contribution of mutant FUS to SG impairment, we characterize FUS interactome in different cell types upon ALS-associated severe mutation, P525L. We show that FUSP525L exhibit increased affinity to H1.2 protein in motor neurons compared to human induced pluripotent stem cells. We also demonstrated that decreasing the levels of H1.2 leads to improved neuronal survival and recovery of delayed SG dynamics. Complementary to these findings, H1.2 overexpression aggravates FUS-ALS phenotype. Our data from FUS-ALS worm model further confirm the findings in motor neurons. Importantly, knockdown of H1.2 worm orthologs leads to decreased FUSP525L aggregation and improves neuro-muscular activity, while H1.2 overexpression exhibits worsening of FUS-P525L aggregation and motility. Since H1.2 is associated with amyloid-beta and alpha-synuclein aggregation, we propose that internally disordered terminal tails of H1.2 might promote FUSP525L phase separation. Targeting H1.2 might lead to therapeutic advancements not only for ALS, but also for other neurodegenerative diseases.

41. Deletion of endothelial TDP-43 impairs retinal angiogenesis and disrupts the vascular barrier in the central nervous system.

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Background: Defects in vascular growth and stability are common features in many pathological processes, including neurodegenerative diseases. The molecular alterations contributing to vascular defects in neurodegenerative disorders are not fully understood. TDP-43 is a DNA/RNA-binding protein that regulates gene expression and its malfunction in neurons has been causally associated with multiple neurodegenerative diseases. Although progress has been made in understanding the functions of TDP-43 in neurons, little is known about its role in endothelial cells (ECs), angiogenesis and vascular homeostasis.

Methods: We generated endothelial-specific and inducible TDP-43 knockout mice and studied the role of TDP-43 in retinal angiogenesis and vascular homeostasis using immunostaining techniques. The molecular mechanisms underlying the in vivo phenotypes were elucidated by knocking down TDP-43 in cultured human ECs.

Results: Deletion of TDP-43 in postnatal ECs results in impaired retinal vascularization due to vessel sprouting defects associated with reduced EC migration and proliferation. In maturing vessels of the central nervous system, loss of TDP-43 results in altered actin cytoskeleton organization, disorganized distribution of cell-cell junction proteins and impaired vascular barrier integrity. and, consequently, hemorrhages and inflammation in the retina, brain and spinal cord. Cultured TDP-43-depleted ECs show reduced stable adherens junctions and altered cell-matrix adhesion sites. Mechanistically, loss of TDP-43 leads to increased actomyosin contraction, preventing proper formation of cell-cell and cell-matrix adhesions.

Conclusions: Our results indicate that TDP-43 is essential for the formation of a stable and mature vasculature and identify endothelial TDP-43 as an important regulator of vascular barrier function, contributing to cell-cell junction integrity.

42. Development of an AAV gene therapy for C9orf72 ALS by targeting the repeat expansion containing C9orf72 transcripts

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Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disorder affecting the upper and lower motor neurons in the brain and corticospinal tract. The most frequent genetic cause of familial ALS is a hexanucleotide GGGGCC repeat expansion in the C9orf72 gene. These expanded repeats are bidirectionally transcribed into repeat containing transcripts, which forms sense and antisense RNA foci. These repeat containing transcripts can also be translated in a non-ATG dependent manner into different dipeptide repeat proteins. Both the RNA foci and dipeptide proteins contribute to disease by toxic gain of function properties.

We are developing an AAV gene therapy targeting the repeat expanded C9orf72 mRNA transcripts (V1 and V3) to inhibit the formation of RNA foci as well as dipeptide proteins using the miQURE® platform. Numerous miQURE constructs were tested in vitro and the most potent miQUREs were selected for in vivo testing in 2 ALS mouse models.

In a C9 BAC transgenic mouse model, we have shown that delivery of AAV-miQURE (AAV-miC9O) resulted in selective silencing of the mutant C9orf72 mRNAs, while preserving the healthy (V2) C9orf72 transcript.

In another ALS model, expression of miQURE targeting mutant C9orf72 mRNA resulted in a substantial decrease in RNAi foci and dipeptide proteins. In addition, a significant improvement in motor and cognitive function could be measured in behavioral assessments in these mice compared to the negative control group.

In conclusion, we have shown strong, selective and sustained lowering of repeat expansion containing C9orf72 transcripts in ALS mice, resulting in reduction of RNA foci and dipeptide protein formation. Furthermore, AAV5-miQURE treatment led to a rescue of the ALS phenotype in mice. These promising data support the advancement of AAV-miQURE for further investigation as a potential treatment for C9orf72-associated ALS.

43. Differential neuronal vulnerability to TDP-43 pathology in a mouse model of amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease characterised by the selective death of motor neurons in the motor cortex, brain stem and spinal cord. Cognitive, extrapyramidal, and sensory impairments have also been described in ALS patients, which suggests additional novel pathophysiological mechanisms beyond the motor system.

A hallmark of ALS is an accumulation of TAR DNA-binding protein 43 (TDP-43)-inclusions in the neuron, which plays a significant role in ALS pathology. Despite TDP-43 being ubiquitously expressed, it remains unclear why neurons are relatively susceptible to the pathology.

Using a neuronal-only overexpressed human TDP-43 mouse model (a severe homozygous genotype was generated by crossbreeding hemizygous overexpressing hTDP-43 mice), we assessed the degree of neuron loss and accumulation of phosphorylated TDP-43 (pTDP-43) throughout the spinal cord, to determine the relationship between neuronal death and pTDP-43 accumulation. As pTDP-43 pathology is driven in all neurons in this mouse model, we aim to advance our understanding of differential neuronal vulnerability to TDP-43 pathology within the spinal cord.

The expected neuronal loss was confirmed in the ventral horn but was also seen in the dorsal horn sensory pathway. Loss was more pronounced in the homozygotes but still significant in hemizygotes. Even though the mouse model studied uses a ubiquitous neuronal driver, Thy1, which results in almost universal labelling of neurons, pTDP-43 accumulation was present in a subset of neuronal nuclei in both dorsal and ventral horns. These accumulations were much more darkly stained in homozygotes and were true at all levels of the spinal cord assessed. Compared to other spinal cord regions and genotypes, the lumber region of homozygous mice showed the highest levels of both neuron degeneration and pTDP-43 accumulation, which suggests a dose-dependent TDP-43 pathology both at a cellular and a population level.

Based on these findings, we suggest that this model can be used to probe differential neuronal vulnerability to TDP-43 over-expression. Techniques such as NanoString sequencing could be used to identify differential pathways and processes in resistant and vulnerable neurons in the ventral and dorsal horn. This may lead to a better understanding of TDP-43 disease pathology and provide valuable insight into neuronal susceptibility in ALS.

44. Differential pathways of astrocyte toxicity converge in common neurotoxicity markers

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Astrocytes are highly specialised glial cells that perform a variety of complex functions to support the health of motor neurons. When co-cultured with murine motor neurons our group has demonstrated that astrocyte-mediated toxicity contributes to, or even drives, motor neuron degeneration under pathological conditions. While astrocyte toxicity is a common ALS phenotype despite a patient's genetic background, we propose that some of the underlying mechanisms contributing to neuron degeneration are unique between patients, while some are common.

Astrocytes derived from three C9ORF72, three SOD1 and three sporadic ALS patients were co-cultured with murine motor neurons with the readout of motor neuron survival establishing a hierarchy of cell line toxicity. RNA-sequencing was performed to identify common as well as unique pathways of astrocyte toxicity.

Pathway analysis was performed with the differentially expressed transcripts for each ALS subgroup and this revealed toxicity-related pathways that were unique to each genotype, such as inflammatory (C9ORF72) and protein processing (sALS) pathways. When the C9ORF72, SOD1 and sALS patient iAstrocytes were analysed together, 127 transcripts were identified that indicate a relationship with cell line toxicity across ALS subgroups. When the gene expression values for these 127 transcripts were plot in the established hierarchy of cell line toxicity, 11 transcripts showed a discerning correlation where the gene expression level was significantly increased in the lines considered most neurotoxic. qPCR analysis of these 11 transcripts confirmed that 8 transcripts could be used as a marker of toxicity. Using single cell RNA-seq from ALS post-mortem tissues, we confirmed that increased expression of two of these transcripts correlates with increased risk of developing ALS.

While unique pathways of astrocyte toxicity were defined between subgroups, we have identified potential biomarkers of astrocyte toxicity that correlate with an increased risk to develop ALS and severity of phenotype.

45. DNA Damage Response Defects are Rescued by Enhancing Chromatin Ubiquitination and DDRNAs Biogenesis in TDP-43 and FUS P-525L ALS Cellular Model System

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Endogenous DNA damage has been reported in motor-neurons (MNs) of ALS patients carrying mutations in the genes encoding for TDP43 or FUS, only recently involved in the DNA damage response (DDR). We published that DDR activation and DNA repair depends on the RNases DROSHA and DICER, cofactors of FUS and TDP43, for the biogenesis of the DNA damage response RNA (DDRNA). We have also reported that the small molecule enoxacin, already shown to ameliorate ALS symptoms, promotes DNA repair by stimulating DDRNA production. Our hypothesis is that neurodegeneration of ALS patients with TDP43 or FUS proteinopathies – present in 97% of all ALS cases – is caused by altered DDR functions.

We observed that FUS and TDP43 cytoplasmic inclusions (CI) reduce DROSHA activity and impair DDRNA biogenesis. This is associated with aberrant and dysfunctional activation of the main DDR kinases ATM and DNAPK, leading to widespread nuclear accumulation of yH2AX, physical DNA damage and loss of cell viability. Treatment with specific small molecule inhibitors of the kinase activity of ATM and DNAPK reduces yH2AX signal in cells with TDP43 and FUS CI, indicating a causative role for such kinases in the observed hyperactivation of DNA damage signalling in TDP43/FUS-proteinopathies. Importanty, enoxacin administration was beneficial in rescuing normal DDR activation in cells with inclusions. We finally extended and validated our findings in a D. melanogaster TDP43 model of ALS, where we observed that the inactivation of ATM or the overexpression of DICER2 counteracts neurodegeneration, further establishing a genetic link between ALS and DDR genes.

46. Dysregulation of the endocannabinoid system in human post-mortem samples from patients with frontotemporal dementia

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The endocannabinoid system (ECS) is a complex cell-signaling system that plays a critical role in regulating several physiological processes such as the maintenance of neuronal homeostasis and integrity. Dysregulation of the ECS has been described in several neurodegenerative disorders, which affects its endogenous neuroprotective role against excitotoxicity, oxidative stress, inflammation, and/or other events involved in neuronal death, then contributing to the disease progression. Indeed, its pharmacological modulation has demonstrated beneficial effects against neurodegeneration. Dysregulation of the ECS has been confirmed in amyotrophic lateral sclerosis (ALS) using animal models and human post-mortem samples, but little information exists on frontotemporal dementia (FTD), which belongs, like ALS, to the same disease spectrum regarding their clinics, genetics, and neuropathology. Our group has recently described changes in some elements of the ECS in a TDP-43-based FTD mouse model, whose pharmacological modulation has been proven to be effective as a neuroprotectant. We are now interested in studying possible changes in ECS elements in human post-mortem samples of FTD patients obtained from two Spanish biobanks. To this end, we first carried out immunohistochemical analyses of common cellular markers to confirm the neuropathological signature of diseased brains (n=10; 5 with FTD-TDP-43 pathology and 5 with FTD-Tau pathology) compared to control cases (n=6). As expected, neuronal loss and reactive gliosis were noticeable in every FTD case. Next, we found these changes were associated with a decrease in CB1 receptor (CB1R) and a subtle increase in fatty acid amide hydrolase (FAAH) immunoreactivity visible in the frontal cortex and hippocampal dentate gyrus of FTD patients as compared to controls. Besides immunohistochemistry analyses, we determined the relative protein levels of CB1R and FAAH enzyme in fresh-frozen brain tissue from the same FTD patients and controls. While no changes were found in FAAH expression, CB1R levels were downregulated in the frontal cortex homogenates, but not in the hippocampus, of all FTD patients. Therefore, as previously described in ALS, the alterations found in patient samples suggest an association of the ECS with FTD pathogenesis. Similarly, its pharmacological modulation may serve to develop a neuroprotective therapy for a disease with poor therapeutic outcome.

47. Early mechanisms of neurotoxicity associated with intercellular spreading of TDP-43 pathology

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ALS is a progressive neurodegenerative disorder that ultimately leads to the loss of motor neurons (MNs). The onset of the disease is triggered by an early pathological event that is uncertain in nature and spreads topographically from a specific point. This ultimately leads to the manifestation of symptoms throughout the neuromuscular system, culminating in paralysis and death. Therefore, we suggest that investigating the early neurodegenerative events in MNs should start with modelling the pathological phenomenon of disease spreading. Our proposal is to examine the secretome of various cell types affected by ALS TDP-43 pathology, such as glia, muscle, and neurons. We believe that these secretomes will induce critical alterations in healthy human MNs, affecting their functional and gene expression levels. Our preliminary studies show that the secretome from patient-derived myotubes induced a clear shift towards a functional hyperexcitability that promptly turns into hypoexcitability and neuronal dysfunction. Hyperexcitability is an early feature common to other neurodegenerative disorders. Remarkably RNA-Seq analysis demonstrates an explicit change in the transcriptomic profile of the neurons that were treated with ALS secretome towards an upregulation in cell-cycle and DNA metabolism-related pathways, resulting in an acquired immaturity-like transcriptome. After examining various RNAseq datasets from ALS neurons, which include those obtained from iPS cells, mouse models, and spinal cord tissue, we have observed a consistent trend. In conclusion, based on our initial findings, it appears that the hyperexcitability gained through exposure to ALS-secretome may be responsible for the immaturity of MN. This could potentially be one of the key early mechanisms behind the neurotoxicity resulting from the intercellular spreading of ALS TDP-43 pathology.

48. Effect of high-fat diet on hippocampal glutamatergic transmission and neuroinflammation in a murine model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most prevalent motor neuron disease characterized by the progressive degeneration of motor functions and other non-motor symptoms, including cognitive deficits. Although the mechanisms by which progressive degeneration and death of motor neurons occurs have not yet been elucidated, it has been described a positive correlation between body mass index and delayed symptoms in ALS patients. Thus, the main aim of this study was to evaluate the impact of a short-term high fat diet (HFD) intake on synaptic plasticity and glutamatergic neurotransmission in the hippocampus of the TDP-43 bigenic mice (NEFH-tTA/tetO-hTDP-43ΔNLS, 'rNLS', mice), also useful in studying frontotemporal lobar degeneration (FTLD). In addition, the mRNA expression levels of receptors relevant to hippocampal glutamatergic transmission (AMPA-1, AMPA-2, NMDA1, NMDA2A, NMDA2B) was analysed by RT-qPCR analysis. Although the role of neuroinflammation in the pathology of ALS is unclear, by confocal imaging we analysed the hippocampus (CA1, CA2, CA3 and dentate gyrus) and motor cortex (M1 and M2) using immunohistochemistry (IHC) analysis, to investigate the effect of HFD consumption on glial response. Our results showed an impairment on Long-Term Potentiation (LTP) and Basal Synaptic Transmission (BST) in rNLS mice regardless of the diet. There were significant effects of genotype in the hippocampal expression profile of AMPA1, NMDA1, NMDA2A and NMDA2B mRNAs. IHC analysis revealed changes of the characteristic features of activated microglia and astrocytes in the brain rNLS mice. Our findings reveal a strong association between aberrant TDP-43 levels and hippocampal function impairment in rNLS mice, however, more evidence-based experimental knowledge is required in order better address the physiological impact of HFD consumption in rNLS mice.

49. Effect of mutations in the C-terminal region of Tardbp in knock-in mice at the phenotypic and molecular level

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TAR DNA-binding protein (TARDBP) is a multifunctional protein involved in RNA metabolism, which has been linked to several neurodegenerative disorders. Mutations in the C-terminal region of TARDBP have been associated with amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The aim of this study was to investigate the effects of C-terminal TARDBP mutations on the phenotypic and molecular level in a knock-in (KI) mouse model.

Here, we studied a new KI mouse model carrying a pathogenic Q331K mutation and another mutation in C terminal region, the M323K mutation. Our results show that the M323K and Q331K mutations in mouse Tardbp exhibit some behavioral changes and led to a significant gain of fucntion in the splicing function compared to wild-type mice. Furthermore, our results show that the M323K and Q331K mutations in Tardbp lead to alterations in stress granule (SG) formation and the mutated Tardbp proteins had an increased tendency to aggregate and a reduced ability to sustain liquid-liquid phase separation (LLPS), which may contribute to the pathogenesis of ALS and FTLD.

Overall, our results demonstrate that the M323K and Q331K mutations in Tardbp have significant effects on splicing function, regulation of SG dynamics and the aggregation potential or the ability to sustain LLPS in the mouse model. Our findings suggest that these mutations may contribute to the pathogenesis of neurodegenerative diseases such as ALS and FTLD. The Tardbp knock-in mice models developed in this study provides a valuable tool for investigating the underlying mechanisms of these diseases and for developing therapeutic strategies.

50. Effects of VIP and the ADNP-derived peptide NAP in the SOD1(G93A) mouse model of ALS: physiological and therapeutic significance

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly affecting motor neurons. The origins of the disease have not been fully elucidated but immune imbalance and neuronal cell death are key components during disease onset and progression. In this study, we evaluate, for the first time, the impact of the VIP neuropeptide system in the SOD1G93A mouse model for ALS. VIP has both neuroprotective and immune regulatory activities. NAP is an octapeptide derived from the VIP-responsive protein activity-dependent neuroprotective protein (ADNP). Unexpectedly, we found that VIP/NAP treatments in prophylactic (8 weeks) or therapeutic (12 weeks) regimes precipitated the disease onset although they reduced the progression rate of the disease hallmarks. The life expectancy in SOD1G93A mice after treatments was not modified. To overcome potential pitfalls due to peptide instability, we used lentiviral-transduced adipose-derived mesenchymal stem cells (AD-MSCs) overexpressing VIP. Although the analysis after cell therapy intervention of the time to disease onset, rates of disease progression and life expectancy showed no differences, we identified VIP responder mice that, remarkably, preserved their balance and motor coordination for more than 200 days. Therefore, our results pointed to the potential of a VIP-based intervention using AD-MSCs, although the underlying factors that makes mice to be responder to VIP delivery by AD-MSCs remains to be explained. Moreover, in order to address the specific role of endogenously produced VIP in ALS, we generated VIP-deficient SOD1G93A mice (null or heterozygous). Notably, while female VIP-deficient SOD1G93A mice showed an increased in the severity of the disease without altering the time of onset, the absence of VIP in male SOD1G93A mice exhibited the opposite effects. Without considering gender differences, the effect of VIP-deficient SOD1G93A mice led to an overall delay in the time of, with an increase in disease severity and without affecting the life expectancy. These results provide unique in vivo evidence that endogenous VIP regulates ALS progression. Moreover, the differential effects observed between exogenous versus endogenous VIP shed light on critical features related to the mechanisms of actions of neuropeptides.

51. Efficacy of Sodium Phenylbutyrate and Ursodoxicoltaurine Combination in Transgenic Mice Displaying Progressive Motor Neuron Degeneration Phenotype

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INTRODUCTION: Endoplasmic reticulum (ER) stress, caused in part by misfolded protein accumulation, and mitochondrial dysfunction play key roles ALS pathology. PFN1 encodes the actin polymerization protein, profilin 1. In vitro/silico and mouse models with ALS-associated PFN1 variants showed increased likelihood of protein misfolding/aggregation, resulting in neuronal death. An oral, fixed-dose combination of sodium phenylbutyrate and ursodoxicoltaurine (PB&TURSO) is hypothesized to reduce neuronal death by simultaneously mitigating ER stress and mitochondrial dysfunction. A transgenic mouse model (Thy1.2-PFN1C71G/Prp-PFN1C71G) was developed expressing mutant human PFN1 displaying a progressive motor neuron degeneration phenotype. We used this model to evaluate whether PB&TURSO conferred a greater therapeutic benefit compared with PB or TURSO alone.

METHODS: Wild-type (WT) or PFN1C71G mice were dosed once daily for 6 weeks (5 d/wk) beginning at age 12/16 weeks with vehicle or 400 mg/kg of of PB, TURSO, or PB&TURSO. Body weight measurements and electromyography recordings of peak compound muscle action potential (CMAP) were collected. Two-way analysis of variance was used to test treatment/genotype effects and identify statistically significant treatment effect differences.

RESULTS: Each treatment group had 8 mice/sex/genotype and were well balanced based on age, sex, genotype, and average body weight per sex. In the cohort initiating treatment at age 12 weeks, vehicle-treated PFN1C71G mice showed a 23.4% peak CMAP reduction (67.7±5.1 mV) versus vehicle-treated WT (88.4±2.3 mV), reflecting modest motor function decline. PB&TURSO-treated PFN1C71G mice showed statistically significant partial rescue of CMAP decline relative to vehicle-treated WT (80.9±3.0 mV; $P<.05$). In the 16-week age cohort, a 52.2% reduction was observed in vehicle-treated PFN1C71G mice (36.9±5.1 mV) versus vehicle-treated WT (77.4±2.5 mV), reflecting major motor function decline. PB&TURSO-treated PFN1C71G mice showed statistically significant partial rescue of this decline (54.6±5.7 mV, $P<.05$). The cohorts treated with PB or TURSO alone did not demonstrate significantly different peak CMAP compared with vehicle-treated controls. Treatment with PB&TURSO conferred a benefit in decreasing motor function decline in a mouse model of neurodegeneration not shown with treatment of PB or TURSO alone, emphasizing the benefit of combination therapy.

52. Elucidating mechanisms underlying FUS aggregation and spreading in neurodegeneration

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Aberrant cytoplasmic accumulation of the misfolded RNA binding proteins, FUS (Fused in Sarcoma) and TDP-43 (Transactive response DNA-binding protein 43) is a nearly universal feature of most amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) cases, the second most common form of young dementia. Both disorders present pathological, genetic and clinical overlap and currently there is no effective treatment to treat either disease. The contribution of FUS aggregation to ALS/FTD pathogenesis remains poorly understood. It is not clear whether there is a correlation between the severity of disease, type of FUS mutation and nature of neuropathological inclusions, nor is it established whether the neuropathology of clinical ALS/FTD reflects the heterogeneity observed in most neurodegenerative diseases.

Our lab generated humanized FUS mice where we focally inject human recombinant mutant FUS fibrils to induce cytoplasmic mislocalization and aggregation of endogenous FUS, which spreads over time throughout the brain of the mice. I will further exploit this newly established in vivo model for focal and temporal FUS aggregation to study human FUS pathology and its temporal dynamics. I will further combine this with innovative cellular systems to gain insights into the cell identities and mechanisms that drive FUS aggregation, cell-to-cell transmission and ultimately toxicity.

53. Elucidating the contribution of the C-terminal sequence of ALS mutated KIF5A in protein aggregation

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease mainly characterised by the loss of upper and lower motor neurons. Patients suffering from this pathology experience a progressive paralysis due to muscle denervation and usually die within 5 years after symptoms onset. Despite the underlying mechanisms responsible for the selective loss of motor neurons being still unclear, several genes with heterogeneous functions have been causally linked to ALS. Recent genomic studies have shown that one such gene is KIF5A, which encodes for a neuronally enriched kinesin involved in protein transport. While mutations in the motor domain of KIF5A are known to lead to hereditary neurodegenerative conditions as Spastic Paraplegia 10 and Charcot Marie Tooth disease, the mutations identified in ALS patients are predicted to alter the mRNA splicing, leading to a frameshift mutation affecting the cargo binding domain. Overexpression of the ALS form of KIF5A triggers the accumulation of cytotoxic protein aggregates and induces apoptosis in HEK cells and primary neurons. Similar aggregates were also identified in human iPSC-derived motor neurons. In this project, we focused on elucidating the contribution of the aberrant sequence in the aggregation process. Our data indicate that protein aggregation and impaired degradation are directly linked not only to mutations occurring within the cargo-binding domain of KIF5A, but also to its interaction with the other protein domains. This represents a crucial alteration characterising the pathobiochemistry of the ALS-KIF5A cases.

54. EST79232 and EST79376, two novel Sigma-1 Receptor ligands, exert neuroprotection on models of motoneuron degeneration

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Motor neuron diseases (MNDs) include sporadic and hereditary neurological disorders characterized by progressive degeneration of motor neurons (MNs). There is no effective treatment to prevent MN death so novel targets for treatment are needed. Sigma-1 receptor (Sig-1R) is a protein enriched in MNs, and mutations on its gene lead to various types of MND. Previous studies have suggested that Sig-1R is a target to prevent MN degeneration. In this study, two novel synthesized Sig-1R ligands, coded EST79232 and EST79376, from the same chemical series, with the same scaffold and similar physicochemical properties but opposite functionality on Sig-1R, were evaluated as neuroprotective compounds to prevent MN degeneration. We used an in vitro model of spinal cord organotypic cultures under chronic excitotoxicity and two in vivo models, the spinal nerve injury and the superoxide dismutase 1 (SOD1)G93A mice, to characterize the effects of these Sig-1R ligands on MN survival and modulation of glial reactivity. The antagonist EST79376 preserved MNs in vitro and after spinal nerve injury but was not able to improve MN death in SOD1G93A mice. In contrast, the agonist EST79232 significantly increased MN survival in the three models of MN degeneration evaluated and had a mild beneficial effect on motor function in SOD1G93A mice. In vivo, Sig-1R ligand EST79232 had a more potent effect on preventing MN degeneration than EST79376. These data further support the interest in Sig-1R as a therapeutic target for neurodegeneration.

55. Excitation/ Inhibition Balance in Amyotrophic Lateral Sclerosis (ALS)

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We believe that vulnerable motor neurons (MNs) experience a reduced excitation before degeneration and that inducing MN excitability contributes to restoring synaptic structures and decreasing disease burden. Hence in the presence of reduced excitation, decreasing inhibitory input to MNs might constitute a viable option to restore a proper balance. In order to test this hypothesis, first inhibition was studied in the ventral horn of the spinal cord of B6SJL-Tg(SOD1*G93A)1Gur/J (mutSOD1) mice, then it was targeted and manipulated at the level of MNs through an AAV9 encoding a GFE3 system. The GFE3 system consists of a nanobody-like protein linked to an E3 ubiquitin ligase that recognizes gephyrin protein and ubiquitinates it leading to its degradation. Our results so far showed changes in inhibitory connections on MNs at presymptomatic stage; there was a decrease in the number of Glycinergic synapses in addition to morphological changes in the GABAergic synapses where we saw an increase in the area of postsynaptic proteins and a decrease in that of presynaptic proteins. However, there were no changes in the number of inhibitory interneurons at presymptomatic stage or end disease stage. Later, inhibition was targeted at the presymptomatic stage through the GFE3 viral vector that was injected into MNs. The GFE3 system led to the removal of gephyrin protein from the MNs which caused a decrease in the number of GABAergic and Glycinergic synapses. It also caused an increase in neuronal activity, as seen by pCREB; and this was proven to be unrelated to changes in excitatory synapses. Moreover, the decrease in inhibition on MNs led to a decrease in disease markers as shown by misfolded SOD, LC3A, and 8OhDG. Therefore, inhibitory connections to MNs are disrupted in mutSOD1 mice, and decreasing inhibitory inputs on MNs in these mice helps ameliorate disease burden.

56. Gene editing reveals ADNP protein (Activity-Dependent Neuroprotective Protein) physiological roles in microglial and its potential implications in neu

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The ADNP protein (Activity-Dependent Neuroprotective Protein) has been identified as an essential factor throughout the embryonic development of the CNS as well as in cognitive function. Some studies have shown the high prevalence of ADNP mutations in certain groups of patients with syndromic autism spectrum disorders (ASD) and aberrant expression of ADNP seems to be related to the development of neurodegenerative diseases such as Alzheimer's or Parkinson's disease. We also have associated ADNP in ALS pathophysiology. Neurodegenerative diseases have a clear neuroinflammatory component. However, the role of ADNP in microglia has not been studied so far, and the molecular microglial mechanisms that operate both in physiological conditions and in pathophysiological alterations are unknown. ADNP SIM-A9 KO microglial cells show key alterations in TANK-binding kinase 1 (TBK1), p38 mitogen-activated protein kinase (MAPK), TDP43 phosphorylation status and defective autophagy/mitophagy mediators as well as mitochondrial real-time metabolic features. Insights in molecular mechanisms involved the BRD4 chromatin reader in microglial cells. Our results are correlated with functional impairments in primary motoneuron spinal cord cultures. These results disclose a physiological role of ADNP as a homeostatic repressor protein in microglia.

57. GEOexplorer: a webserver for gene expression analysis and visualization

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Gene Expression Omnibus (GEO) is a database repository hosting a substantial proportion of publicly available high throughput gene expression data. Gene expression analysis is a powerful tool to gain insight into the mechanisms and processes underlying the biological and phenotypic differences between sample groups. Despite the wide availability of gene expression datasets, their access, analysis, and integration are not trivial and require specific expertise and programming proficiency. We developed the GEOexplorer webserver to allow scientists to access, integrate and analyse gene expression datasets without requiring programming proficiency. Via its user-friendly graphic interface, users can easily apply GEOexplorer to perform interactive and reproducible gene expression analysis of microarray and RNA-seq datasets, while producing a wealth of interactive visualisations to facilitate data exploration and interpretation, and generating a range of publication ready figures. The webserver allows users to search and retrieve datasets from GEO as well as to upload user-generated data and combine and harmonise two datasets to perform joint analyses. GEOexplorer, available at https://geoexplorer.rosalind.kcl.ac.uk, provides a solution for performing interactive and reproducible analyses of microarray and RNA-seq gene expression data, empowering life scientists to perform exploratory data analysis and differential gene expression analysis on-the-fly without informatics proficiency.

58. Harnessing Transcriptomic Signals for Amyotrophic Lateral Sclerosis to Identify Novel Drugs and Enhance Risk Prediction

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. This study integrates the latest ALS genome-wide association study (GWAS) summary statistics with functional genomic annotations with the aim of providing mechanistic insights into ALS risk loci, inferring drug repurposing opportunities, and enhancing prediction of ALS risk and clinical characteristics.

Methods: Genes associated with ALS were identified using GWAS summary statistic methodology including SuSiE SNP-based fine-mapping, and transcriptome- and proteome-wide association study (TWAS/PWAS) analyses. Using several approaches, gene associations were integrated with the DrugTargetor drug-gene interaction database to identify drugs that could be repurposed for the treatment of ALS. Furthermore, ALS gene associations from TWAS were combined with observed blood expression in two external ALS case-control datasets to calculate polytranscriptomic scores and evaluate their utility for prediction of ALS risk and clinical characteristics, including site of onset, age at onset, and survival.

Results: SNP-based fine-mapping, TWAS and PWAS identified 117 genes associated with ALS, with TWAS and PWAS providing novel mechanistic insights. Drug repurposing analyses identified five drugs significantly enriched for interactions with ALS associated genes, with directional analyses highlighting α-glucosidase inhibitors may exacerbate ALS pathology. Additionally, drug class enrichment analysis showed calcium channel blockers may reduce ALS risk. Across the two observed expression target samples, ALS polytranscriptomic scores significantly predicted ALS risk (R2 = 4%; p-value = 2.1×10-21).

Conclusions: Functionally-informed analyses of ALS GWAS summary statistics identified novel mechanistic insights into ALS aetiology, highlighted several therapeutic research avenues, and enabled statistically significant prediction of ALS risk.

59. hnRNPH in nuclear G4C2 foci and cytoplasmic stress granules of C9ORF72 ALS

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The most common genetic cause of ALS is hexanucleotide G4C2 repeat expansion in the first intron of C9orf72. One of the hallmarks is the formation of RNA foci in the nucleus, G4C2 foci, which contain aberrant repeat transcripts and sequester a variety of RNA-binding proteins (RBP). hnRNPH is a member of a large protein family of RBPs involved in the regulation of alternative splicing, mRNA stabilization, transcription, and translation. In many neurodegenerative diseases, including ALS, increased oxidative stress characterised by the formation of stress granules (SG) is a concomitant pathological factor. In ALS brain tissue, hnRNPH colocalizes with nuclear RNA G4C2 foci, whereas under cellular stress conditions, it is localized in cytoplasmic stress granules. Sequestration of hnRNPH in insoluble RNA aggregates correlates with dysregulation of splicing and may contribute to neurodegeneration. Our goal was to reveal the domains of hnRNPH that determine its localization in G4C2 foci and stress granules. Nuclear foci share a group of interacting proteins with stress granules and their simultaneous presence in ALS neurons could have further pathological implications. We designed a series of hnRNPH1 protein constructs based on its domain structure and introduced mutations into individual qRRM domains to disable their RNA-binding activity. Quasi (q)RRM2 and qRRM3, but not qRRM1, were sufficient for localization of hnRNPH in stress granules. Localization of hnRNPH in G4C2 foci was independent of the RNA-binding activity of any individual qRRM domain. Using RBDmap, we demonstrated that the putative ZnF domain of hnRNPH may have RNA-binding activity. Surprisingly, hnRNPH protein localized to G4C2 foci even after the removal of the RNA-binding activity of the qRRM and ZnF domains. This result suggest that RNA-binding activity may not be the only driving force for the sequstration of hnRNPH into the G4C2 foci associated with C9orf72 ALS.

60. Identifying compounds for drug repurposing in ALS using a TDP-43 mutant mESC-derived motor neuron model

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Drug repurposing, where clinically approved drugs are re-examined for use in other disorders, is an advantageous approach that overcomes challenges faced by traditional drug discovery methods. To identify compounds that warrant further downstream analysis, phenotypic readout assays in a relevant model system are required. Our group has previously developed a BAC transgenic mouse embryonic stem cell-derived motor neuron (mESC-MN) model that expresses TDP-43-WT or TDP-43-M337V at low levels. TDP-43-M337V mESC-MNs demonstrate dysregulated stress granule characteristics and reduced viability in response to oxidative stress compared to controls. Here, we use mESC-MN cellular viability in response to oxidative stress as a screening platform to identify compounds that may be repurposed for the treatment of ALS. Subsequently, we examine the effects of lead compounds on additional phenotypes in mESC-MNs and zebrafish models of ALS. Mouse ESCs were expanded as embryoid bodies and differentiated to form motor neurons. mESC-MNs were treated with the PHARMAKON 1600 compound library then incubated with sodium arsenite at 0.5 mM for 1 hour to induce oxidative stress. Cellular viability was assessed by fluorescence intensity following incubation with media containing resazurin (10 mg/ml) for 24 hours. For all downstream analysis in mESC-MNs, cells were treated with compounds at 2.5 mM for 24 hours. To investigate the effects of compounds on axonal transport, mESCs were incubated with MitoTracker, then imaged with live cell microscopy. To examine the effects of compounds on stress granule formation, mESC-MNs were treated with sodium arsenite at 0.5 mM for 1 hour prior to fixation. The effects of compounds on axonal length in zebrafish was examined at 30 hours post fertilisation (hpf) in controls, mutant TDP-43 overexpression (OE) and C9orf72 knockdown (KD) fish. Treatment with the FDA-approved compound library and downstream validation identified six lead compounds that improved viability in TDP-43M337V mESC-MNs in response to oxidative stress. Compounds were found to rescue impaired stress granule formation in TDP-43-M337V mESC-MNs, and improve axon length in TDP-43 and C9orf72 zebrafish models of ALS. No clear class effects of lead compounds were identified, suggesting these compounds may act through a non-canonical pathway. Given this, in future work we aim to investigate the mechanisms through which these compounds exert their neuroprotective effects.

61. Identifying Forkhead Box Q1, as a novel regulator of ferroptosis

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Ferroptosis, an iron-dependent form of necrotic cell death characterized by phospholipid peroxidation and metabolic constraint. Ferroptosis has emerged to play an important role in cancer biology as a mean to contribute to a number of pathologies. Cells respond to ferroptotic stimuli by regulation of selenoproteins, including the key regulator of ferroptosis phospholipid hydroperoxide-reducing enzyme glutathione peroxidase 4 (GPX4). Though, the underline mechanisms and signaling pathways of the ferroptosis cell death remains still relatively unknown, however, it is crucial to understand the pathophysiological role of ferroptosis and how it may be exploited for the treatment of cancer. Genome-wide screening was performed and a transcription factor named FOXQ1 was identified as a promising factor involved in the regulation of cellular ferroptosis sensitivity. The transcription factor FOXQ1 is a member of forkhead proteins that emulate important function in biological process during development and tumorigenesis. A very limited studies have investigated the role of the FOXQ1 in human cancer. In my thesis, enforced expression cloning approach was used to elucidate the factor and cell-autonomous mechanisms that underlines the regulation of ferroptosis in the given cell line. Preliminary data now shows increased sensitivity to ferroptosis in FOXQ1 overexpressing cells compared to WT cells. To analyze the relevance of FOXQ1 in cell cycle, endogenous FOXQ1 was knocked out by using CRISPR/Cas9 technology. SgRNA mediated depletion of FOXQ1 reduced invasive ability of the cells. FOXQ1 deletion resulted in lower proliferation rates of cells and cell death compared to those which were transduced with a control non-targeting vector.

To demonstrate the effect of FOXQ1 overexpression on the ferroptosis regulation, a panel of stably overexpressing cancer cell lines have been generated and challenged with different ferroptosis inducing agents. Cell proliferation, ferroptotic markers and senescence markers has been monitored at several time points. Furthermore, this knowledge will help to reveal the role FOXQ1 in ferroptosis as a potential therapeutic target for the development of anticancer therapies and to identify novel transcriptional downstream targets of FOXQ1 which may impact on ferroptosis.

62. IFB-088, a multifunctional drug candidate targeting the major ALS cellular pathophysiological mechanisms

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IFB-088 (icerguastat or Sephin1) is a first-in-class, brain penetrant, orally available small molecule which prolongs the Integrated Stress Response pathway. This cellular mechanism aims to restore protein and cellular balance to prevent cell death. By prolonging eIF2a phosphorylation, IFB-088 gives stressed cells more time to correct protein imbalances and clear protein aggregates. IFB-088 have been shown to be efficacious in several animal models of neurodegenerative diseases, type 1A and 1B of Charcot-Marie-Tooth disease and SOD1G93A-mediated ALS.

As proteostasis is not the only cellular pathway dysregulated in ALS, the neuroprotective effect of IFB-088 has been evaluated on glutamate excitotoxicity, , protein aggregation and axonal regeneration.

The effects of IFB-088 on motoneuron viability, mitochondrial ROS, TDP-43 mislocalisation were evaluated in primary SOD1G93A rat motoneurons. The effects of IFB-088 were evaluated on motoneuron viability and TDP-43 expression from spinal cord of SOD1G93A mice treated orally with IFB-088 at 4 or 8mg/kg/day for 12 weeks starting at 8 weeks of age. The impact of IFB-088 on axonal regeneration was evaluated in nerve crush on WT mice treated twice a day by oral gavage with IFB-088 at 1 or 3 mg/kg/day for 3 weeks.

Upon ER stress, IFB-088 increases eIF2a phosphorylation leading to a prolongation of protein translation attenuation and an increase of cell survival Treatment with IFB-088 progressively increases eIF2a phosphorylation, tends to reduce TDP-43 in triton insoluble fraction and improves motoneurons survival in spinal cord of the SOD1G93A mice. IFB-088 improves TDP-43 mislocalisation and cell survival following glutamate stress in primary SOD1G93A rat motoneurons. IFB-088 improves primary rat cortical neuron survival following NMDA intoxication by antagonising the NMDA receptor through the binding of IFB-088 to NR2B subunit leading to a reduction of calcium influx. IFB-088 reduces mitochondria ROS following glutamate application in primary SOD1G93A rat motoneurons. In a nerve crush experiment, IFB-088 administrated at 3mg/kg/day improves CMAP amplitude, myelin thickness and g-ratio improving axonal regeneration.

Unlike FDA approved ALS drugs, IFB-088, currently in Phase 2 clinical trial (NCT055080742), has the unique potential to improve several cellular pathways dysregulated in ALS, such as proteostasis, glutamate excitotoxicity, oxidative stress, TDP-43 cellular mislocalisation and axonal regeneration.

63. Imaging the spinal cord neurodegeneration of the TDP-43-A315T ALS mouse model: relationship between MRI and TDP-43 aggregates

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Few studies showed Magnetic Resonance Imaging (MRI) alterations in the spinal cord of ALS patients (1), and suggest their relation with disease duration and progression (2,3). TDP-43 aggregates, the hallmark of ALS, were also shown to be related with disease progression (4). So far, no study reported the relation between MRI alterations and the occurrence of TDP-43 aggregates. Here, we performed behavioral and MRI analysis in the TDP-43-A315T mice model and correlated such analysis with the presence of TDP-43 aggregates, before and after symptoms' onset. Female transgenic TDP-43-A315T (TDP) and wild-type C57BL/6J (WT) mice were used. Weight and motor function were assessed weekly, and MRI was performed at 3, 6 or 9 months (n=5 for each group). Mice were euthanized immediately after MRI for samples collection. At 3 months-old, there was no difference regarding body weight (p=0.4) or grip strength (p=0.06) between groups. TDP-43-A315T mice start to show a decline in body weight and motor function at 4 months-old. At 6 and 9 months, TDP-43-A315T mice presented significant gait impairment, decline in tail position, body weight and hindlimb grip strength. MRI analysis of spinal cord showed no differences at 3 and 6 months but the diffusion coefficient decreased at 9 months. Western blot analysis of the spinal cord revealed an increase in total, aggregated and phosphorylated TDP-43 at 9 months. WB analysis for cellular markers of astrogliosis, microgliosis and neuronal loss are ongoing. The absence of MRI alterations at 3 months correlates with a normal motor function in analyzed mice. As described in the literature and confirmed in this study, symptoms' onset for the TDP-43-A315T start around 4 months of age. Relation of MRI alterations and TDP-43 aggregates could represent a novel biomarker for ALS. Furthermore, they could be used in preclinical studies or as an outcome in clinical trials to follow treatment efficacy against TDP-43 aggregates.

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64. Immune-mediated myogenesis and acetylcholine receptor clustering promote a slow disease progression in ALS mouse models

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Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease with high variability in the speed of progression even in cases with a defined genetic cause such as superoxide dismutase 1 (SOD1) mutations. SOD1G93A mutation on mice with distinct genetic backgrounds (C57 and 129Sv) show consistent differences in speed of disease progression resembling what is observed in ALS patients. We recently hypothesized that the difference in the peripheral neuromuscular system rather than the extent of spinal motor neuron loss reflects the phenotypic difference between these two mouse models. Therefore, we redirect our attention to the skeletal muscle as an early component of ALS pathogenesis, aiming to discover the molecular mechanisms contributing to the distinct phenotypes and to identify factors underlying fast and slow disease progression. In this work, we compare the functional, morphological and molecular profiles of the gastrocnemius muscle (GCM) from these two SOD1G93A mouse strains at the pre-symptomatic and onset stage of the disease. Data collected clearly defined the extent of NMJ stability and muscle regeneration as a discriminator between rapidly and slowing progressing ALS mice. Notably, the slow-progressing mice, despite the premature denervation and muscle atrophy, activate different compensatory mechanisms including the expression and clustering of the AChR, myogenesis and immune system response, which are able to delay the onset and progression of their symptoms. On the contrary, the fast-progressing mice that are unable to activate these responses exhibit a rapid decline of muscle force. This study highlights a set of key genes and molecular pathways indices of fast or slow disease progression, which may prove useful in identifying potential disease modifiers responsible for the heterogeneity of human Amyotrophic Lateral Sclerosis, which may provide new opportunities to hamper the disease progression.

65. Impact of parthenolide on primary microglial cells and motor neurons derived from mutant SOD1-G93A mice

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Over the last two decades, research on the role of microglial activation has become increasingly important in the field of neurodegenerative diseases. Dysregulation of microglial properties and function appears to be involved in the pathogenesis of the fatal degenerative motor neuron disease Amyotrophic Lateral Sclerosis (ALS). Microglial cells, the immune cells of the nervous system, can have different phenotypes, which can be pharmacologically manipulated. The sesquiterpene lactone parthenolide, found mostly in the flowers and leaves of the feverfew (Tanacetum parthenium) appears to be a promising neuroprotective drug candidate capable of interfering with microglial properties. It showed positive treatment effects in animal models of neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. We now intended to investigate the impact of parthenolide on primary microglia derived from mutant SOD1-G93A mice.

Effects of parthenolide we assessed via measurement of mRNA levels of microglia phenotype markers in primary microglia cells derived from mutant SOD1-G93A mice. In addition, we analyzed the impact of treatment-induced modulation of microglial phenotype on motor neurons in co-cultures of primary motor neurons and microglial cells via immunocytochemistry.

Our preliminary results show that parthenolide reverses LPS-induced changes the reactive state of mutant SOD1-G93A- primary microglia cells based on mRNA expression levels of tumor necrosis factor alpha, inducible nitric oxide synthase and interleukin-1 beta. Besides that, we also found a positive impact on primary motor neurons derived from SOD1-G93A-mice.

As there is no cure for ALS so far, it is important to study drugs with potential impact on known disease mechanisms. Parthenolide seems to have effects on factors and pathways involved in ALS onset and progression.

66. Impaired cytoplasmic dynein mediated transport of signalling endosomes in motor neurons dysregulates ERK1/2 signalling pathway

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Cytoplasmic dynein 1 is responsible for the majority of intracellular microtubule-based retrograde transport. Signalling endosomes, formed during receptor endocytosis, link dynein to processes of cell signalling, such as EGF/EGFR and BDNF/TrkB pathways. Their degradation is required to terminate the induced signal after ligand-receptor binding. Dynein trafficks signalling endosomes to lysosomes, terminating receptor signalling. Signalling pathways activate a variety of different proteins such as kinases – including ERK1/2. These kinases modulate a range of functions such as survival, proliferation, or migration, in part through changes in gene expression. As a result, alterations in signalling pathways may have knock-on effects on this process, leading to potentially pathogenic changes in gene expression. Previous research indicates that impaired dynein function through the Legs-at-odd-angles (Loa) mutation (DYNC1H1F580Y) in mice leads to delayed trafficking of signalling endosomes, with increases in ERK1/2 phosphorylation following EGF and BDNF stimulation in Mouse Embryonic Fibroblasts (MEFs) and motor neurons respectively, representing an alteration to the typical signalling pathway.

We hypothesise that, due to the axonal length of motor neurons, deficits in retrograde transport may substantially alter signalling pathways in these neurons and lead to potentially pathological changes in gene expression in both motor neurons and skeletal muscle. This may lead to gradual cell death after development, or negatively impact development during gestation through changes in neuronal migration or axonal extension and contribute to the vulnerability of motor neurons and musculature in ALS. Thus, we aim to elucidate the effect of dynein impairment on gene expression directly.

Through Nanopore sequencing, we are investigating changes in gene expression when dynein is impaired in Loa MEFs and have found over 300 significantly dysregulated genes ($p<0.05$), combined with up to 3-fold increases in ERK1/2 phosphorylation, indicating that dynein may play a role in regulating gene expression through timely degradation of receptor signalling, modulating signal pathway activity. Multiple genes show biological relevance to motor neuron and muscle health, and we are investigating these cell types to further understand how pathological changes in gene expression arising from impaired dynein function may contribute to the progression of motor neuron degeneration.

67. Impaired response to hypoxia in a physiologically relevant Drosophila model of C9orf72 FTD/ALS.

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Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two clinically overlapping neurodegenerative diseases, characterised by cognitive and motor impairments respectively. The most common genetic cause of FTD/ALS is a G4C2 hexanucleotide repeat expansion in the C9orf72 gene, with affected individuals carrying 100s to 1000s of repeats. There are three proposed mechanisms leading to neurodegeneration downstream of the expansion: 1) haploinsufficiency, 2) formation of toxic RNA foci by repeat RNA and 3) non-canonical translation of the expansion to produce 5 toxic dipeptide repeat proteins (DPRs): poly-GR, PR, GA, AP and GP. While all three of these mechanisms may contribute to disease, DPRs have been identified as the most prominent driver of neurodegeneration. However, specific mechanisms underlying DPR toxicity remain unclear. Hypoxic exposure and genetic variation in hypoxia genes have previously been identified as risk factors for ALS, while impaired hypoxia signalling has been identified in FTD/ALS patients and models. An impaired hypoxia response has also been demonstrated to be sufficient to cause neurodegeneration in mice. However, hypoxia and impaired hypoxia signalling have never been linked to C9orf72 FTD/ALS. Here, we model C9orf72 FTD/ALS in Drosophila by expressing DPRs of a physiologically relevant length in the fly nervous system. Exposing these flies to hypoxia by incubation in a hypoxia chamber, we demonstrate an aberrant response to hypoxia in our model. Using behavioural assays, we demonstrate that flies pan-neuronally expressing GR(1000) exhibit a delayed stupor response to hypoxia. We also show by RT-qPCR that GR(1000) flies exhibit differential transcription of hypoxia signalling genes in response to hypoxia exposure. Our findings indicate that poly(GR) disrupts the ability of the Drosophila nervous systems to sense and/or respond to hypoxia. This disruption provides a possible mechanism of neurodegeneration in C9orf72 FTD/ALS.

68. IMPROVING PATHOLOGICAL PROTEIN ANALYSIS BY FLOW CYTOMETRY AND IMMUNOFLUORESCENCE IN ALS.

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ALS is a lethal neurodegenerative disease characterized by the loss of motor neurons, that triggers a loss of muscle tone leading to a progressive paralysis and death. TDP-43 is the main affected protein in the disease which has important functions in the nucleus, such as stabilizing RNA and transporting mRNA. At pathological conditions this protein is aberrantly aggregated in the cytoplasm, phosphorylated by other proteins and the nucleo-cytoplasmic homeostasis is lost. Current treatments are not effective against ALS disease and therefore research is much needed.

There are several drug candidates which aim is to restore TDP-43 homeostasis, such as protein kinase inhibitors IGS2.7 or Tideglusib. However, in order to measure whether these drugs are being effective, there is a need to develop techniques to measure TDP-43 homeostasis. We have employed a biobank of ALS lymphoblasts with which we aim at measuring in a quantifiable and rapid methodology TDP-43 homeostasis. We have performed the studies in lymphoblasts from healthy controls, ALS patients with and without pharmacological treatment.

Preliminary studies showed that we could measure the levels of these proteins before and after drug treatment, and these results could be confirmed by immunofluorescence. The next step is to tag more than two targets simultaneously in order to better understand the pathological mechanisms underlying this disease in single cell studies.

69. In vivo functional validation of the ALS causative p.E696K missense mutation in TBK1.

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Deleterious mutations in the autophagy regulator TANK1-binding kinase 1 (TBK1) cause ALS. Unlike other ALS causative TBK1 missense mutations, p.E696K selectively abolishes the interaction of the TBK1 protein with the autophagy adaptor protein optineurin (OPTN). The p.E696K mutation triggers ALS most likely through a partial loss-of-function. Our group has generated Tbk1E696K knock-in mice carrying the p.E696K mutation leading to a block in the autophagic flux, while kinase activity is preserved. The mutant mice show age-dependent ALS-like motor and neuropathological phenotypes, such as the increased accumulation of cytosolic p62 positive inclusions. In this study, we apply these models for: 1) a better understanding of the cellular and molecular dysfunction linked to the onset and progression of mutant TBK1 (mTBK1) pathology, and 2) functionally validating the impact of mTBK1 on the accumulation of toxic proteins. To this end, we have investigated how mTBK1 dysregulates transcriptional profiles of different cell-types in the spinal cord of the Tbk1E696K knock-in mice at pre- and early-symptomatic stages by single nuclei RNA-sequencing. In parallel, we have generated double knock-in mice carrying a mutant Huntingtin (mHTT) allele in combination with the Tbk1E696K knock-in. Based on previous evidence that HTT is phosphorylated by TBK1, we have analyzed the impact of Tbk1E696K on accumulation of mHTT protein inclusions in different brain regions. In particular, we have found early transcriptional changes in astrocyte and microglia profiles. Moreover, we found a consistent increase in the number of mHTT and p62 positive inclusions in the double mutant mice, supporting that the p.E696K mutation can impair clearance of aggregation prone proteins. These studies suggest the potential application of these models to dissect the cell-, stage-, and disease-specific function of TBK1, and to possibly identify shared across disease mechanisms.

70. "Increased ADAM 10/17 activity in an animal model of ALS: rationale for targeting ADAMs as potential therapeutic target?"

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Background.

ADAMs comprise a large family of transmembrane metalloproteases responsible for ectodomain proteolytic cleavage (shedding) of membrane-tethered proteins. ADAM17 was originally identified as the major enzyme for TNF-alpha release, and ADAM10 could compensate for this function. ADAM 10/17 activity is increased in proinflammatory conditions and has already been implicated in the pathogenesis of Alzheimer's disease and Multiple Sclerosis. However, information on potential involvement of ADAM10/17 in ALS is still scarce.

Aims.

Our goal is to elucidate whether alterations of ADAM10/17 protein expression, distribution and/or enzymatic activity could play a role in ALS. Moreover, ADAM10/17 can be released in circulation, and we explored CSF/blood ADAM10/17 activity as a potential ALS biomarker.

Methods.

We performed immunohistochemistry and western blot analyses in spinal cord districts at different disease stages in the SOD1.G93A transgenic (TG) rat model of ALS. In parallel, we measured ADAM10/17 activity in spinal cord homogenates, in CSF and blood.

Results.

We highlighted a selective increase of ADAM10/17 immunoreactivity in motor neurons of TG animals at the onset of the disease; as the disease progresses to the late stages, ADAMs are upregulated in glial cells in the white and gray matter. ADAM10/17 enzymatic activity, measured in tissue homogenats, was increased at the symptomatic stage of the disease. Finally, we measured increased levels of ADAM10/17 activity only in the CSF of TG at the symptomatic stage, whereas in the blood was negligible.

Discussion.

Although our data are still preliminary, we highlighted alterations of ADAM10/17 distribution at the early symptomatic stage of the disease. We are currently investigating the correlation between ADAMs and some of their substrates, such as TNF-alpha or GPNMB implicated in the disease; in parallel, we are exploring selective pharmacological ADAMs inhibitors as a potential therapeutic approach.

71. Insights into KIF5A-related pathways to neurodegeneration

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KIF5A is a neuron-specific kinesin involved in anterograde axonal transport. It comprises a head domain for microtubule binding and ATP hydrolysis, a stalk domain for dimerization, and a tail domain for autoinhibition and cargo/adaptor binding. Mutations occurring in these KIF5A domains are associated with distinct neurodegenerative diseases (NDs), including ALS, but the bases of such genotype/phenotype heterogeneity still have to be fully elucidated. To investigate the molecular mechanisms underlying KIF5A-dependent NDs, we analysed the biochemical behaviour of five disease-associated KIF5A mutants (R17Q, R280C, R864X, N999VfsX39, C975VfsX73) affecting the different protein domains.

In NSC-34 cells, we overexpressed the ALS-linked N999VfsX39 and the Charcot-Marie-Tooth-related R864X mutants and found that they mainly localise within neurites instead of showing the diffused cytoplasmic distribution of overexpressed WT KIF5A. However, the two mutants differed, since R864X KIF5A was found to be diffused within neurites, while N999VfsX39 KIF5A formed p62-positive puncta. Notably, both mutants sequestered WT KIF5A within neurites and showed partial co-localisation with mitochondria, well-established KIF5A cargos.

In SH-SY5Y cells, cycloheximide chase evidenced a lower stability for the N999VfsX39 and the spastic paraplegia-associated R17Q mutants compared to WT KIF5A, hinting at an altered protein turnover. Accordingly, proteasomal blockage resulted in N999VfsX39 and R17Q KIF5A accumulation into detergent-insoluble inclusions, suggesting that these mutants are degraded by the ubiquitin-proteasome system and that proteostasis impairment might promote their deposition into aggregates.

Interestingly, the aberrant biochemical behaviours of N999VfsX39 KIF5A were recapitulated to a more severe extent by the novel C975VfsX73 variant, linked to neonatal intractable myoclonus (NEIMY) and sharing the last portion of its abnormal C-terminal tail with the ALS mutant. Indeed, C975VfsX73 KIF5A accumulated into large, p62-decorated inclusions that sequestered WT KIF5A and lacked interaction with mitochondria, highlighting a phenotypic similarity between the ALS- and NEIMY-related mutants.

Together, our results indicate that both unique and shared molecular mechanisms underpin KIF5A-dependent NDs.
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72. Integrative proteomics highlight presynaptic alterations and c-Jun misactivation as convergent pathomechanisms in ALS

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Background: One of the main obstacles to the development of efficient therapeutic approaches for ALS is the variability of its genetics. Indeed, ALS-related genes are linked to a variety of biological functions, including protein transport, autophagy, DNA damage repair and RNA metabolism. Nevertheless, all of the pathological changes connected to various ALS genes eventually result in the loss of motor neurons, indicating the existence of common but unknown pathomechanisms. Our previous work showed the synapses as a critical component actively involved in ALS, raising the possibility that synaptic abnormalities may represent a common pathogenic hallmark across the ALS spectrum.

Methods: We performed the first in-depth investigation of the synaptic proteome of hiPSC-derived motor neurons carrying mutations in the C9orf72, FUS, TARDBP and SOD1 genes. In order to highlight a widespread malactivation of certain biological pathways in ALS, we also examined the phospho-proteome of motor neurons carrying same mutations. Finally, we conducted drug testing to identify a molecule that would have a beneficial impact by correcting the detected impairments.

Results: Our combinatorial approach revealed vesicle release machinery abnormalities as a common pathomechanism in ALS. Phosphoproteomic investigation provided insight on the shared pathobiochemistry of ALS by linking the presynaptic vesicular phenotype to a buildup of cytotoxic protein aggregates and to the activation of the transcription factor cJun, which promotes apoptosis. Importantly, sub-chronic docosahexaenoic acid treatment of our iPSC-derived motoneurons had a neuroprotective impact by successfully restoring the abnormalities discovered by our multidisciplinary approaches.

Conclusions: This study presents strong evidence for the central and convergent function of the synaptic microenvironment in the ALS spinal cord and reveals a potential therapeutic target that prevents degeneration in a diverse cohort of human motoneuron cells.

73. Interactomes of wt and C-terminally truncated FUS

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Neuronal degeneration has been recognized as a predominant driver of disability and disease progression in central nervous system diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Attempts to develop treatments for these disorders have not yet revealed clinically successful. The aggregation of RNA binding proteins (RBPs) has been recognized as a hallmark pathological feature in these disorders, defining them as proteinopathies. Fused in sarcoma (FUS), normally a nucleus residing RBP, is known to aggregate into physiological granules and pathological inclusions, which can impair cell homeostasis leading to neuronal cell death. The mutations in FUS that alter its C-terminal nuclear localization signal (NLS) proved autosomal dominant in ALS and showed to disrupt its nucleo-cytoplasmic shuttling and increase its cytoplasmic localization. Since protein interactors of FUS and the exact signaling pathways involved in cytoplasmic toxicity of FUS remain unknown, we aimed to identify the interactomes of FUS and FUSdNLS (lacking NLS) proteins overexpressed in a model cell line using BioID2 proximity labeling. This technique harnesses the ability of the enzyme biotin ligase (birA) to biotinylate proximal endogenous proteins. To this end we prepared constructs of the FUS and FUSdNLS conjugated to BioID2 enzyme by a flexible linker and transiently expressed them in HEK293T cells. We cleared the biotinylated proteins from cell lysates and analyzed them by mass spectrometry. Bioinformatic analyses of proteomic data identified interaction candidates involved in RNA processing and degradation, protein translation and various signal transduction pathways. Selected interactions were validated by pull-down assay and cell co-localization analyses in vitro In vitro analyses demonstrated FUS to interact with NUDT21 and decrease its nuclear expression, whereas NUDT21 interaction to be abolished with FUSdNLS, possibly having downstream effects on 3‘RNA cleavage and polyadenylation processing. The interactome differences between FUS and FUSdNLS, provide detailed insight into FUS function most likely relevant to disease, that could be targeted in therapeutic interventions.

74. Intracerebroventricular delivery of AAV9-based gene therapy selectively transduces motor neurons in the spinal cord when delivered to neonatal mice

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Amyotrophic lateral sclerosis (ALS) is a devastating, and ultimately terminal, motor neuron disease that is characterised by progressive loss of upper and lower motor neurons. ALS has several known genetic etiologies and therefore the therapeutic potential for gene transfer and correction using viral vectors is becoming increasingly appreciated and explored. One of the key challenges when delivering vectors is ensuring that they successfully reach and transduce lower motor neurons in the spinal cord. This is particularly important when screening and trialing novel gene therapies to assess their neuroprotective potential in vivo; for vectors to exert robust effects, we require them to reach the spinal cord, transduce the motor neuron and express at high enough levels to affect detectable biological change. There are several routes of administration intro cerebrospinal fluid that are already well-described, however direct comparisons of different routes for targeting the central nervous system remain limited in mice. Here, we aim to compare the distribution of virus after administration of adeno-associated virus 9 (AAV9)-CMV-green fluorescent protein (GFP) when delivered either intracisterna magna (ICM) or intracerebroventricular (ICV) spaces. Using qPCR to assess viral copy number in different tissues and immunohistochemical approaches in brain and spinal cord, we demonstrate that when delivered before postnatal day 3, unilateral ICV administration effectively transduces motor neurons in the spinal cord. We show further amplification of motor neuron transduction can be achieved through bilateral ICV injection across 2 days. We also confirm that ICM delivery provides effective cerebellar transduction, however spinal cord distribution was limited. We note that whilst intramuscular injection effectively transduced muscle, there was a lack of evidence to support retrograde transduction of motor neurons. Overall, this has important preclinical implications, as it highlights the importance of selecting the most appropriate administration route for more targeted use of novel gene therapies.

75. Investigating Purine Metabolism in C9orf72 ALS

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Background: Astrocytes are key neuronal support cells, vital in understanding motor neuron degeneration in ALS. Previous data has identified purine metabolism dysfunction in C9orf72 induced neuronal progenitor cell derived astrocytes (C9-iAstrocytes), specifically loss of the enzyme adenosine deaminase (ADA). To ascertain the full effect of the C9orf72 hexanucleotide repeat expansion (C9-HRE) on purine metabolism we characterised this pathway in C9-iAstrocytes. We also utilised C9-HRE models of pathogenicity to understand the mechanisms responsible for loss of ADA and investigated targeted lentiviral gene therapy to restore ADA expression.

Methods: We measured the expression and activity of ADA, inosine and uric acid output, and expression of purine enzymes upstream and downstream of ADA in C9-iAstrocytes. ADA lentiviral gene therapy was used to restore ADA levels in C9-iAstrocytes, and the effect on purine metabolism was assayed. Purine enzyme expression was further explored in C9orf72 knockout HeLa, N2a transduced with 38x sense (G4C2) or 39x antisense (C4G2) repeats and HeLa transfected with 36x glycine-alanine (poly-GA), glycine-arginine (poly-GR) or proline-arginine (poly-PR) dipeptide repeat proteins (DPRs).

Results: ADA activity, inosine output and uric acid levels were reduced in C9-iAstrocytes. Downregulation of the anti-inflammatory purine enzyme ecto-5'-nucleotidase (CD73) and upregulation of the purine salvage enzyme hypoxanthine guanosine phosphoribosyl transferase (HGPRT) was also observed. C9orf72 knockout in HeLa cells did not alter purine enzyme expression, whilst ADA levels were significantly reduced in both C4G2 N2a and poly-PR transfected HeLa cells. Moreover, CD73 levels were reduced in poly-PR transfected HeLa cells, though upregulation of HGPRT was not observed in any C9-HRE model. ADA gene therapy restored ADA expression without altering CD73 or HGPRT levels.

Conclusions: The C9-HRE induces several aberrations in purine metabolism, which may in part be caused by poly-PR aggregation, as C4G2 and poly-PR expressing models exhibited reduced levels of ADA and CD73. HGPRT upregulation may be induced via a separate mechanism currently under investigation. CD73 loss is likely not caused by loss of ADA, as ADA gene therapy had no effect on CD73 and is more likely the direct effect of poly-PR expression. Further mechanistic analysis of the pathway is underway.

76. Investigating the Role of NEK1 in ALS.

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Heterozygous loss-of-function (LOF) mutations in NEK1 are a cause of familial and sporadic ALS, and nonsynonymous and LOF mutations in the NEK1-interacting protein CFAP410 are associated with increased ALS risk. Both NEK1 and CFAP410 are involved in DNA damage repair pathways, but how their loss causes ALS remains unclear.

The endoplasmic reticulum (ER) and mitochondria are closely associated organelles that are physically connected at specialised regions of the ER known as mitochondria associated membranes (MAMs). These inter-organelle contact sites have been shown to regulate several physiological processes that have been implicated in ALS including autophagy, calcium homeostasis, and synaptic function. Reduced ER-mitochondria coupling and signalling has been reported in multiple forms of ALS, including ALS associated with mutations in SOD1, TDP-43, FUS, Sigma-1R, and C9orf72.

Our data indicate that NEK1 and CFAP410 are part of a novel signalling complex at ER-mitochondria contacts that regulates interactions between ER and mitochondria, with loss of NEK1 or CFAP410 leading to reduced contact between the organelles. Such a complex could provide a direct link between DNA damage, ER-mitochondria contacts, autophagy, and neurodegeneration.

77. INVESTIGATING VARIANTS IN NUP50 AS RISK FACTORS FOR AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is the major adult onset motor neuron disease with a significant genetic contribution. We have previously conducted a discovery transcriptome-wide association study (TWAS) on the largest genome-wide association study (GWAS) cohort to date (29,621 cases and 120,971 controls [1]) and identified 30 loci associated with ALS [2]. Interestingly, decreased expression of NUP50, a gene encoding a nuclear pore basket protein, was associated with ALS in TWAS. To date, NUP50 is thus the first direct genetic link related to nucleocytoplasmic transport, which is suspected to be affected in ALS. We reported at least nine NUP50 variants present in ALS patients which are predicted to be pathogenic by in silico analysis [2]. Importantly, knocking down NUP50 led to increased neuronal death associated with p62 and nucleoporin inclusions in cultured neurons [2]. However, the precise molecular mechanism by which NUP50 alteration may contribute to ALS is unknown. As the NUP50 variants identified were all located in the binding domains to importin-α and nucleoporin 153 (NUP153), which are proteins involved in nuclear import of macromolecules, we hypothesized that the variants may therefore affect the nucleocytoplasmic transport function of NUP50. Thus, ongoing work in a neuronal cell culture model is currently being carried out to investigate whether these variants impact 1) the normal localization and arrangement of the NUP50 protein at the nuclear membrane, 2) the efficiency of nucleocytoplasmic transport via the use of reporters, 3) the interaction of NUP50 with known interactants and 4) the localization of proteins (i.e. TDP-43) known to be mislocalized in ALS pathophysiology. Taken together, these first results will shed light on how NUP50 alteration may confer risk to developing ALS, potentially via nucleocytoplasmic transport deficits, which are already considered to be a contributing factor to the disease.

78. LncRNAs in muscle of SOD1G93A ALS model mice: potential as biomarkers and intervention targets.

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The lack of sensitive and specific biomarkers for diagnosis and prognosis, together with the need of a better understanding of the mechanisms leading to ALS, represent major challenges in ALS, which currently delays diagnosis and treatment implementation. In the search for possible molecules that throw new light on this issue, lncRNAs seem to be promising candidates for their regulatory role in a multitude of processes involved in this neurodegenerative disease, such as RNA metabolism, myogenesis, apoptosis or proteostasis.

This work aims to elucidate the expression profile of 12 lncRNAs in skeletal muscle, a tissue that has been shown to contribute to the pathogenesis of ALS, throughout the different stages of the disease using SOD1G93A murine model.

Results obtained showed that 9 of the 12 lncRNAs studied were differentially expressed in SOD1G93A versus WT mice. This expression profile varied depending on sex and age. This finding is consistent with the different disease progression observed in males and females of this murine model of ALS, as well as its different frequency in patients. Among the 9 altered lncRNAs found, the expression levels or progression of 4 of them in muscle biopsies were shown to correlate with animal survival.

Finally, we conclude that the results collected in this work suggest that lncRNAs may have an important role in the pathogenesis and development of ALS, in addition to a possible value as biomarkers.

79. Loss of CHK1 contributes to DNA damage accumulation in cells bearing WT TDP-43 and FUS P525L cytoplasmic inclusions

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. Mutations in the TARDBP (TAR DNA Binding Protein) and FUS (Fused in Sarcoma) genes, which encode for TDP-43 and FUS proteins resectively, have been identified in a subset of patients. A hallmark of ALS is the aggregation of TDP and FUS proteins co-localizing with stress granule (SG) markers in the cytoplasm of affected motoneurons. In addition, accumulation of DNA damage has been observed in patients. We use transient overexpression of WT TDP-43 or FUS P525L in HeLa cells to study the impact of cytoplasmic inclusions (CIs) on genome integrity and DNA damage repair (DDR) signalling. We found that the presence of CIs co- localizing with SG markers correlates with accumulation of DNA damage, defects in the DDR and cessation of cell proliferation. Of note, these cells show an aberrant activation of ATM, the apical kinase of the DDR signalling cascade. Chemical inhibition of ATM but not ATR (the apical kinase of the replication stress response) reduces DNA damage accumulation in cells bearing CIs. Cells with CIs show very low incorporation of BrdU and stain negative for cyclin A, excluding replication stress as the cause of DNA damage. This observation makes our system a relevant model to study a neurodegenerative disease since also neurons are non-dividing cells. We observed that the two factors CHK1 and ASF1A, recently involved in DDR signalling and DNA repair in non-replicating cells, are down-regulated in cells bearing CIs. We found that transient overexpression of CHK1 and ASF1A reduces the accumulation of DNA damage in cells with CIs and partially rescues defects in the DDR. Importantly, we observe increased TDP-43 cytoplasmic inclusions that co-localise with stress granules markers also in motoneuron progenitors derived from a sporadic ALS patient. In these cells we also observe an accumulation of DNA damage.

80. Loss of HSF1 and HSC70 protection in spinal motor neurons contributes to the onset of ALS pathogenesis

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Amyotrophic lateral sclerosis (ALS) patients suffer adult onset paralysis due to motor neuron (MN) degeneration. However, one of the most mysterious aspects of ALS pathogenesis is that ALS-associated genes like FUS are ubiquitously expressed including during development. We speculate that induced pluripotent stem cell (iPSC)-derived neurons harbor a molecular mechanism protecting against ALS pathogenesis that is lost from aging patient MNs. To identify this candidate mechanism, we analyzed FUS behavior in iPSC-derived neurons. In response to stress, mutant FUS is rapidly incorporated into aberrant stress granules (SGs). However, we unexpectedly found that, instead of forming aggregates and degenerating, iPSC-derived neurons efficiently disassembled aberrant SGs via HSF1-mediated expression of heat shock proteins, including HSP70. In contrast, immunofluorescence revealed MNs in post-mortem ALS spinal tissue manifest severe dysregulation of HSF1 as well as decreased HSC70, the cognate form of HSP70 in correlation with FUS pathology. Using iPSC-derived neurons, we demonstrate that inhibition of HSF1 or HSC70 profoundly affects SG dynamics and neurodegeneration. Experiments using Drosophila models complement these findings. Importantly, over-expression of HSC70 rescued FUS mislocalization as well as degeneration of iPSC-derived neurons with mutant FUS. Similar results were obtained for C9orf72-ALS, indicating a central role for HSC70 and the HSF1-mediated expression of chaperons such as HSP70 in protecting MNs from ALS pathology in multiple disease subtypes. Our results also suggest that gene therapy vectors expressing specific heat shock proteins such as HSC70 could be an effective therapeutic strategy to protect MNs against ALS pathogenesis.

81. MAPK/MAK/MRK overlapping kinase (MOK) regulates microglial inflammatory/type-I IFN responses via Brd4 and is involved in ALS

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Neuroinflammation is an early and recurrent feature in Amyotrophic lateral sclerosis (ALS) and microglial neurotoxic responses are considered to have a key role in disease onset and progression. However, the mechanisms underlying microglial signalling and dysregulated responses remain incompletely understood. To shed light into this question, we used MOK-KO cells and primary microglial and organotypic cultures, as well as the SOD1(G93A) model for ex vivo and in vivo studies combined with a variety of tools including confocal immunofluorescence, flow cytometry, RNA-Seq, ChIP and proteomics. Our results reveal that MAPK/MAK/MRK overlapping kinase (MOK), with unknown physiological substrate/s, displays an immune function by controlling inflammatory and type-I IFN responses in microglia which are detrimental to primary motor neurons. Moreover, we uncover the epigenetic reader bromodomain-containing protein 4 (Brd4) as the first molecule regulated by MOK, by promoting Ser492-phospho-Brd4 levels. We further demonstrate that MOK regulates Brd4 functions by supporting its binding to cytokine gene promoters, therefore enabling innate immune responses. Remarkably, we show that MOK levels are increased in ALS spinal cord, particularly in microglia, and that administration of a chemical MOK-inhibitor to ALS model mice is able to suppress microglial activation and delay disease onset, indicating a pathophysiological role of MOK kinase in ALS and neuroinflammation.

82. MCH neurons in ALS: vulnerability and connectivity

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Altered energy metabolism and weight loss are the major non-motor symptoms that precede the onset of amyotrophic lateral sclerosis (ALS) for years and predict patients' survival. In ALS patients and pre-symptomatic gene carriers increased metabolism was linked to atrophy of hypothalamus, a key regulator of energy balance. However, neural mechanisms driving metabolic impairment in ALS remain largely unexplored.

Here, we aimed to identify vulnerable hypothalamic neuropeptidergic populations and to investigate their specific networks. Hypothalamic neuronal subtypes were assessed in hypermetabolic SOD1G93A mice using in situ transcriptional profiling. We demonstrate that neuronal numbers are preserved in pre-symptomatic mice when body weight is maintained. In contrast, several neuronal populations degenerate in symptomatic stage of the disease when mice rapidly lose weight. Among those, melanin-concentrating hormone (MCH) neurons, that promote food intake, appear to be the most affected with a loss of 40%, highlighting their clinical importance in the context of weight lost.

Next, to distinguish primacy between neuronal loss vs. network impairment we mapped the brain-wide, monosynaptic projections to MCH neurons. To ensure MCH-specific connectivity we used retrograde tracing based on the modified rabies and Cre-inducible helper viruses co-injected into hypothalamus of transgenic Mch-Cre;SOD1G93A and Mch-Cre;WT mice. While whole-brain inputs are similar between genotypes, local-hypothalamic projections to MCH neurons (particularly from zona incerta) are lost early in pre-symptomatic mice. With disease progression, in symptomatic mice spreading of circuit impairment is detected by loss of inputs from another hypothalamic nuclei and by alteration of extra-hypothalamic, long-range inputs to MCH neurons (particularly from cerebral nuclei). Principal component and hierarchical cluster analysis reveal that altered input patterns from hypothalamic and cerebral nuclei to MCH are sufficient to segregate animals into groups according to their genotype. Thus, MCH-specific connectome undergoes disease-related re-modelling in ALS mice, prior to MCH degeneration, weight deficit and disease onset.

Ongoing experiments will characterize MCH inputs and set further in vivo functional manipulation of both MCH and projection neurons using in-house designed chemogenetic tools in order to improve neuronal survival and define their contribution to ALS and therapeutic potential.

83. Metabolic profiling of fibroblasts derived from healthy donors and Amyotrophic Lateral Sclerosis patients bearing the p.G376D mutation in the TARDBP g

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Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease characterized by the loss of upper and lower motor neurons (MN)s (Rowland LP et al., 2001). To date, more than 150 distinct genes have been found to be associated with ALS, among which TARDBP plays an important role. It encodes for TDP-43, a versatile RNA/DNA binding protein involved in RNA-related metabolism: the increase of the cytoplasmic TDP43 concentration leads to cytoplasmic inclusion formation that causes impairments in RNA processing, global mitochondrial imbalance (Moujalled D. et al., 2017) and an augmented oxidative stress (Zuo X. et al., 2021).

In this study we enrolled patients bearing the p.G376D mutation in the TARDBP gene, at different disease stages (early and late), and healthy relatives, with the same mutation but manifesting no symptoms (asymptomatic).

This cohort gave us the unprecedent opportunity to investigate alterations in the energy metabolism of ALS associated genes mutant cells, to better understand their role in disease progression.

We performed a comprehensive metabolic profiling of primary fibroblasts: our preliminary data suggested that patients' fibroblasts have an imbalance in the oxidative stress versus antioxidant defense system, while asymptomatic and healthy volunteers still rely on glutathione as protective mechanisms against ROS production.

Abundant evidence has revealed a prominent role for mitochondrial dysfunction in the pathogenesis of ALS (Cozzolino M. et al., 2012) although the underlying mechanism is still not clear.

So, we exploited the Seahorse technology to in depth assess the mitochondrial functionality (Perciballi et al., 2022). Moreover, we used complementary biochemical and biomolecular assays to evaluate mitochondrial oxidative stress load and cell distribution.

Our study suggests that TARDBP mutations might lead to alterations in the energy metabolism that may match the disease stage.

Further studies are required to understand if metabolic differences between asymptomatic versus symptomatic mutant individuals may be used as protective mechanisms or as early prognosis markers.

84. Mitochondrial genome study in blood of maternally-inherited ALS cases

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ALS is a heterogeneous disease in which different factors act in combination with a genetic predisposition. As one of these factors, mitochondrial phenotypes have been associated with ALS. This study addresses the question of whether homoplasmic (total mitochondrial genome of a sample is affected) and/or heteroplasmic mutations (wildtype and mutant mitochondrial DNA molecules coexist) might play a role in familial ALS. Blood was drawn from familial ALS patients with a maternal pattern of inheritance according to their pedigrees, which was compared to blood of ALS patients without maternal association and age-matched controls. In two cohorts, we analyzed the mitochondrial genome from whole blood or isolated white blood cells and platelets using a resequencing microarray (Affymetrix MitoChip v2.0) that is able to detect homoplasmic and heteroplasmic mitochondrial DNA mutations and allows the assessment of low-level heteroplasmy. We identified a significant increase in homoplasmic ND5 mutations, a subunit of respiratory chain complex I, in whole blood of ALS patients with maternal inheritance. This effect was more pronounced in patients with bulbar onset. Heteroplasmic mutations were significantly increased in different mitochondrial genes in platelets of patients with maternal inheritance. No increase of low-level heteroplasmy was found in maternal ALS patients. Our results indicate a contribution of homoplasmic ND5 mutations to maternally-associated ALS. Therefore, it might be conceivable that specific maternally-transmitted rather than randomly acquired mitochondrial DNA mutations contribute to the disease process. This stands in contrast with observations from Alzheimer's and Parkinson's diseases showing an age-dependent accumulation of unspecific mutations in mitochondrial DNA.

85. Mitochondrial TSPO overactivation via ERK1/2 cascade correlates with impairment of respiration and mitophagy in a mouse model of ALS

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Amyotrophic lateral sclerosis (ALS) is the most common neurological disorder affecting the motor system and causes the progressive degeneration of upper and lower motor neurons in brain stem and spinal cord. Although the mechanisms leading to the selective death of motor neurons are heterogeneous and not fully understood yet, dysfunction of mitochondria and the loss of mitophagy, a specific form of autophagy aimed at recycling irreversibly damaged organelles, are emerging as crucial events in the onset of ALS and, more generally, of all neurodegenerative diseases.

Mitochondria represent the energy supply stations of eukaryotic cells, producing ATP via oxidative phosphorylation (OXPHOS). Furthermore, they participate in a variety of additional biochemical and bioenergetic pathways, such as phospholipid biosynthesis, calcium homeostasis and regulation of cell death and survival. However, swollen and vacuolated mitochondria, characterized by morphological, ultrastructural and bioenergetic alterations, were observed in many ALS models and patients. With the aim of further investigate this aspect, we analyzed the mitochondrial functionality in spinal cords of symptomatic transgenic mice expressing the human SOD1 G93A mutant, a well-established and widely used model of ALS. Here, we found that the impairment of motor abilities correlates with a significant reduction of mitochondrial respiration, precisely the oxygen flows linked to ATP production and maximal capacity, as a result of the partial inhibition of respiration linked to OXPHOS complexes I and II. Notably, this not depends from a downregulation of the relative OXPHOS protein subunits expression, nor from a reduction of the overall mitochondrial mass, suggesting that dysfunctional organelles may accumulate in motor neurons of transgenic mice.

To explore this hypothesis, we looked at the Translocator Protein (TSPO), a small mitochondrial multi-drug binding protein whose upregulation was recently linked to the loss of mitophagy in a Parkinson's disease model. We found a similar TSPO overexpression in the spinal cords of ALS transgenic mice exclusively, that inversely correlates with the expression of Atg12, a positive regulator of mitophagy. Mechanistically, TSPO upregulation associates with the overactivation of ERK1/2 cascade that, in turn, induces TSPO gene transcription via STAT3.

Overall, our findings set out TSPO as a key regulator of mitochondrial homeostasis in ALS.

86. Modeling C9ORF72 DPR pathology using optogenetics.

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Proteinaceous inclusions consisting of aggregated, insoluble dipeptide-repeat proteins (DPRs), arising from the hexanucleotide repeat expansion of C9ORF72, are a hallmark pathology of the most common familial ALS subtype, C9-ALS. Despite the proposed pivotal role of DPRs in toxicity and hence disease pathogenesis, this hallmark pathology has not been efficiently recapitulated in cellular models. Indeed, asides from poly-GA which is highly aggregate-prone, 4 of the 5 known DPRs fail to aggregate and form inclusions, typically displaying a diffuse distribution pattern when expressed in cultured cells. However, aggregation propensity of DPRs may be crucial for their pathogenic effect. It is therefore vital to mimic this property of DPRs to model the disease more accurately. To address this gap, in the current project, we aimed to generate cellular models with optogenetically-controlled phase separation/aggregation of DPRs. We generated constructs for the expression of poly-PR, -GP and -GR tagged with Cry2olig, a Cry2 variant with a high oligomerisation propensity, and a fluorescent tag for live imaging. We then investigated the light-inducible aggregation of these DPRs in stable cell lines using time-lapse automated confocal imaging (Opera Phenix) under several imaging paradigms. We achieved intranuclear and cytoplasmic aggregate induction for poly-PR and -GP, respectively, upon blue light exposure. Aggregation of both DPR species was initially reversible, although with repetitive stimulation in longer time-course experiments (up to 12 hours) we observed the persistence of aggregates. Importantly, poly-PR aggregates were stable upon cell lysis and could be purified for proteomic profiling. Furthermore, we found that although poly-GR does not form similar prominent, readily detectable cytoplasmic aggregates under the stimulation paradigms tested, higher-resolution imaging showed that this DPR species undergoes distinct cytoplasmic fibrillization instead. Overall, our cellular models recapitulate the patterns of DPR aggregation detected in human postmortem tissues and are amenable to downstream biochemical analyses and potential drug screening applications.

87. Modulating the gut dysbiosis by dietary intervention in a murine model of ALS.

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Sequencing of the human genome has greatly improved the understanding of our genetic variability and also helped the investigation of etiopathogeny of diseases. Despite this, causes of many diseases of multifactorial nature such as Amyotrophic Lateral Sclerosis (ALS) remain largely unknown and there is no effective treatment that could slow down the degenerative process in patients. Alterations in the gastrointestinal tract function and metabolism have been observed in ALS animal models, some of which are also present in patients (doi: 10.1016/j.nbd.2018.10.007). Although these alterations may partly be associated with lowered food consumption as a consequence of the disease progression, recent evidence suggests that this could also be caused by dysbiosis of the gut microbiota. Preliminary studies in our group using Nanopore sequencing and qPCR suggested that the ratio of the two predominant bacterial populations, Firmicutes and Bacteriodetes, in stool samples is altered in symptomatic SOD1G93A mice, which is in line with previous studies (doi: 10.1038/s41586-019-1443-5). Furthermore, dietary butyrate increases the abundance of some members of Firmicutes population in this model (doi: 10.1016/j.clinthera.2016.12.014), highlighting the possibility of a beneficial dynamically modulation of the gut microbiota using dietary interventions. The main objective of this study aims to investigate the modulatory and beneficial effect of an antioxidant compound in transgenic SOD1G93A mice that has been probed to preserve the gut barrier, increase the immune cells and reduce bacterial translocation in vivo during inflammatory states. For this purpose, this compound was orally administered in 20 transgenic SOD1G93A mice. Wild type mice (n=20) and non-treated transgenic SOD1G93A mice (n=20) were included as control groups. In all the groups littermates male and female mice were considered. This compound greatly improved the locomotor phenotype of SOD1G93A mice during the disease progression, thus ameliorating the disease progression in the animals, probably in a troponin-mediated manner. In addition, treated SOD1G93A mice also showed a stable weight curve during the disease progression, whereas the untreated transgenic group presented a sharp decay from 107 days old, typical for this model. These findings could foster a relatively rapid translation to clinical trials in ALS patients, which is of particular relevance in motor neuron and neurodegenerative diseases.

88. Mono-methylation of arginine 293 of TDP-43 and its function in translational control

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The methylation of the amino acid arginine in proteins is a post-translational modification (PTM) that can, similar to phosphorylation, change the function and/or localization of proteins. We already validated the finding of multiple proteome-wide screenings that have consistently reported mono-methylation of arginine 293 (R293) of TDP-43 and revealed that the protein arginine methyltransferase 1 (PRMT1) is responsible for this PTM.

R293 of TDP-43 is highly conserved and the center of a hotspot of mutations that are causative for ALS. While we found that ALS-associated mutations in close proximity to R293 lead to decreased methylation, we could not detect substantial effects of this PTM on the solubility or localization of TDP-43, neither under basal nor under stressed conditions. However, proximity-based biotinylation assays (BioID) followed by mass spectrometry of methylation-mimicking and -deficient variants revealed that this PTM regulates the interaction of TDP-43 with ribosomes. Moreover, we found that decreased methylation of TDP-43 increases its association with ribosomes leading to increased overall protein translation. Interestingly, despite being less methylated, ALS-related variants of TDP-43 showed less ribosome association and induced decreased translation indicating that ALS mutations dominate the effect of the methylation. Nevertheless, our recent finding that increased calcium concentration activates PRMT1 and consequently increases the methylation of wildtype TDP-43 suggests a role of this PTM in sporadic ALS which is currently under investigation.

89. Neurofilament accumulations in Amyotrophic Lateral Sclerosis patients' motor neurons impair axonal initial segment integrity.

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Neurofilament (NF) levels in patient' fluids have recently emerged as the prime biomarker of Amyotrophic Lateral Sclerosis (ALS) disease progression, while NF accumulation in MNs of patients is the oldest and one of the best pathological hallmark. However, the way NF accumulations could lead to MN degeneration remains unknown. To assess NF alterations and study the impact on MNs, we have compared MNs derived from induced pluripotent stem cells (iPSC) of patients carrying mutations in C9orf72, SOD1 and TARDBP genes, the three main ALS genetic causes. We show that in all mutant MNs, light NF (NF-L) chains rapidly accumulate in MN soma, while the phosphorylated medium/heavy NF (pNF-M/H) chains pile up in axonal proximal regions of C9orf72 and SOD1 MNs. In more mature MNs also excitability abnormalities were observed. We demonstrate that the integrity of the MN axonal initial segment (AIS), an axonal sub-compartment crucial for excitability and polarity, is impaired in the presence of pNF-M/H accumulations in C9orf72 and SOD1 MNs. We establish a strong correlation between these pNF-M/H accumulations, an AIS distal shift, increased axonal calibers and modified repartition of sodium channels. The results expand our understanding of how NF accumulation could dysregulate components of the axonal cytoskeleton and disrupt MN homeostasis. With recent cumulative evidence that AIS alterations are implicated in different brain diseases, preserving AIS integrity could have important therapeutic implications for ALS.

90. Neuroimmune characterization of aged mice with optineurin insufficiency

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Optineurin is a multifunctional ubiquitin-binding adaptor protein involved in regulation of various cellular processes, such as inflammatory signalling and autophagy. Its mutations were found in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) spectrum disorder. ALS and FTD result in neuronal death, neuroinflammation and proteinopathy in the brain and/or the spinal cord. In this study, we utilized a mouse model carrying a patient-like C-terminal truncation in optineurin (Optn470T). We have previously shown a disbalanced cytokine and chemokine expression profiles upon lipopolysaccharide stimulation in Optn470T mice and primary Optn470T macrophages and microglia. However, young Optn470T mice showed no microgliosis and no overt neurological phenotype. Since one of the major risk factors for chronic immune disbalance and neurodegenerative disease is ageing, we tested if it would act as a second hit for triggering ALS/FTD-like neuropathology in Optn470T mice. We assessed aged mice for motor coordination and cognitive abilities and found no differences between the wild-type (WT) and Optn470T mice up to two years of age. To test if neuropathology precedes motor and/or cognitive impairments, we analysed the spinal cord and the brain sections by immunofluorescence for the presence of neurodegeneration, neuroinflammation and TAR DNA binding protein 43 kDa (TDP-43) aggregation. We found signs of ageing, such as lipofuscin accumulation and increased astrocyte and microglial activation at two years of age. However, the ageing phenotype did not differ between the genotypes. Similar astrocytosis and microgliosis suggested a similar cytokine and chemokine expression, which was further corroborated by cytokine array analysis of the spinal cords and the brains of Optn470T mice. Finally, we found preserved motoneuron numbers and predominantly nuclear TDP-43 in the spinal cords and the brains of both WT and Optn470T mice at one and two years of age. Thus, our results showed that a double hit of optineurin insufficiency and aging was insufficient to precipitate ALS/FTD-like disease in mice. This is similar to several other mouse models carrying human ALS/FTD-linked loss-of-function mutations and suggests that additional stimuli are required to elicit neuropathology in these models.

91. Neuromuscular junction denervation and terminal Schwann cell loss in the hTDP-43 overexpression mouse model of amyotrophic lateral sclerosis (ALS)

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Aims: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with complex aetiology. Despite evidence of neuromuscular junction (NMJ) denervation and 'dying-back' pathology in models of SOD1-dependent ALS, evidence in other genetic forms of ALS is limited by a lack of suitable animal models. TDP-43, a key mediator protein in ALS, is overexpressed in neurons in Thy1-hTDP-43 WT mice. We therefore aimed to comprehensively analyse NMJ pathology in this model of ALS.

Methods: We assessed expression of TDP-43 over several time points during disease progression via western blotting. Immunohistochemistry techniques, alongside NMJ-morph quantification, were used to analyse motor neuron number, neuromuscular junction denervation status and terminal Schwann cell morphology. We also performed survival curves and TDP-43 expression level comparisons between two mouse background strains.

Results: We present a time course of progressive, region-specific motor neuron pathology in Thy1- hTDP-43 WT mice. Thy1-driven hTDP-43 expression increased steadily, correlating with developing hindlimb motor weakness and associated motor neuron loss in the spinal cord with median survival of 21 days. Pronounced NMJ denervation was observed in hindlimb muscles, mild denervation in cranial muscles but no evidence of denervation in either forelimb or trunk muscles. NMJ pathology was restricted to motor nerve terminals, with denervation following the same time course as motor neuron loss. Terminal Schwann cells were lost from NMJs in hindlimb muscles, directly correlating with denervation status and confirming loss of glial support to these synapses.

Conclusions: Thy1-hTDP-43 93 WT mice represent a severe model of ALS, with NMJ pathology/denervation of distal muscles and motor neuron loss, as observed in ALS patients. This model therefore provides an ideal platform to investigate mechanisms of dying back pathology, as well as NMJ targeting disease-modifying therapies in ALS.

92. Neuromuscular ultrasound as preclinical diagnostic biomarker in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis

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Translation of novel therapeutic approaches from in vivo models of Amyotrophic lateral sclerosis (ALS) into humans has proven difficult over the past decades. There is a lack of biomarkers for monitoring disease progression in mouse models. High frequency ultrasound of nerve and muscle has emerged as a readily accessible, non-invasive tool for diagnosis and follow-up of ALS in humans. We hypothesize that neuromuscular ultrasound might be a valuable diagnostic tool in the SOD1G93A mouse model to monitor disease progression non-invasively.

Ultrasound was performed on the sciatic nerve, biceps femoris (BFM) and gastrocnemius (GM) muscles of SOD1G93A transgenic mice at different disease stages. Nerve cross section (CS), nerve cross sectional area (CSA), muscle thickness, opening angle and echo intensity (EI) were evaluated and set in the context of established parameters: weight, motor performance testing (rotarod), neve conduction studies (motor amplitude (sMAP) of GM) and histological data.

CS of the sciatic nerve and opening angle of the GM were significantly smaller in transgenic mice compared to wild types starting from the age of 7 weeks. Thickness and EI of the BFM became significantly different at the age of 13 weeks. sMAP was significantly smaller in transgenic animals starting from 7 weeks. Transgenic animals further had significantly lower weight starting from 10 weeks, while rotarod performance became significantly reduced at 16 weeks. Sonographic signs of muscle atrophy and CS of the sciatic nerve were positively correlated with motor neuron loss in the anterior horn of the spinal cord (Nissl stain). Sonographic CS and CSA of the sciatic nerve further correlated positively with axon diameter, fiber diameter and myelin thickness. Thickness of the BFM correlated positively with axon and fiber diameter as well as the ratio of normal/abnormal fibers, while EI of the BFM correlated negatively with the ratio of normal/abnormal fibers.

Our results indicate that motor neuron loss and muscular atrophy can be measured by ultrasound in the SOD1G93A mouse model. Ultrasonographic changes in nerve and muscle morphology become apparent at the same time as or even precede alterations in established behavioural tests and histological hallmarks of the disease. Neuromuscular ultrasound may therefore be a promising, non-invasive diagnostic marker to monitor effects of novel therapeutics in preclinical studies.

93. No title

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Loss of function and/or expression of members of the fragile X protein (FXP) family comprising the RNA-binding proteins FMR1, FXR1 and FXR2 has repeatedly been linked to several neurodegenerative diseases. Strikingly, in amyotrophic lateral sclerosis (ALS), as well as in Alzheimer's and Parkinson's disease, available evidence indicates disturbed FXP function/expression early in the course of the respective disease, or even in the pre-symptomatic stage, preceding or even contributing to the deposition of pathogenic aggregates. However, there is a strong bias in studying FMR1, and consequences at the protein level induced by the loss of expression of the FXPs are largely unknown. Therefore, in this study, we aim at elucidating and comparing cellular pathways and mechanisms affected by loss of the individual FXPs. Using CRISPR/Cas9-edited knockout cell lines, analyses of total and insoluble proteomes revealed multiple shared and unique functions of these proteins. In the total proteome, especially knockout of FXR2 led to the dysregulation of ALS-related proteins. Increased amounts of chaperones, as well as of components of the proteasome and the autophagic machinery in the insoluble proteomes indicated involvement of the FXPs, directly or indirectly, in protein folding and/or clearance. Knockout of FMR1, but not of FXR1 or FXR2, additionally led to defects in stress granule assembly. However, so far, we could not detect increased aggregation of TDP-43, FUS or SOD1 in these cells, neither of the wildtype proteins nor of ALS-related variants. Therefore, loss of a single FXP alone is likely not sufficient to induce aggregation of ALS-related proteins. Nevertheless, our results indicate at least contribution of the FXPs to pathogenic protein aggregation in neurodegeneration, and identification of additional factors required is ongoing.

94. Nonclinical evaluation of the efficacy of Neural Stem Cells intracerebroventricular transplant as a possible treatment for ALS

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Preclinical studies suggest that neural stem cells (NSCs) treatments might antagonize neurological diseases progression (Rota Nodari et al., 2010; Willis et al., 2020). Among others, we showed that hNSCs transplantation in the spinal cord of SOD1G93A rats extends animals' survival, reduces the main ALS histopathological markers and prevents motor neurons degeneration in the transplanted site (Zalfa et al., 2019). Phase I Clinical Trials (NCT01640067, NCT01348451) demonstrated the safety and feasibility of this approach, also showing a transitory delay of the decline of the ALS- FRS-R (Feldman et al., 2014; Mazzini et al., 2019), however the patient cohort was too small to draw final conclusions. Cell dose escalation by increasing the number of spinal cord injections is limited due to the backbone destabilization required by the surgery (NCT01730716) (Glass et al., 2016).

We are evaluating intracerebroventricular (icv) delivery of hNSCs, as an effective approach to increase cell dosage, favoring a broader spread of transplanted cells and putative secreted healing factors throughout the motor neuraxis by exploiting the CSF circulation and possibly target also the motor cortex. Several studies point to the role of upper motor neuron degeneration in ALS pathogenesis, thus making them suitable therapeutic targets (Özdinler et al., 2011; Thomsen et al., 2018).

We evaluated the safety and biodistribution of icv transplantation (either 300,000 or 1x106 hNSCs/mouse) in immunodeficient mice. Our data show that the transplant is well tolerated and not tumorigenic after 6 months, even at the highest dosage. Of note, hNSCs adhere to the ventricle wall and extensively migrate along the ventricles reaching the central canal of the cervical spinal cord. We observed also cells dispersed throughout the brain thus suggesting that hNSCs can migrate from the injection site into the parenchyma.

The same dosages were tested in SOD1G93A mice by using a transient immunosuppression protocol.

Our preliminary data suggest that the treatment might delay the decline of motor performances. However, cell survival is not optimal thus, at this stage, the reduced sample size prohibits any definitive conclusion.

We are currently evaluating hNSCs survival and efficacy by prolonging the immunosuppression regimen, in order to consolidate our observations and evaluate behavioral and histological parameters to confirm the therapeutic potential of our strategy.

95. Noradrenaline deficiency as a driver of cortical hyperexcitability in amyotrophic lateral sclerosis

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A unifying feature of both sporadic and familial forms of ALS is cortical hyperexcitability, which precedes symptom onset, negatively correlates with survival, and is sufficient to trigger both UMN and LMN degeneration in rodent. Using electrocorticography in the Sod1G86R and FusΔNLS/+ ALS mouse models, we demonstrate that cortical dysfunction in ALS also manifests by a deficit in theta-gamma phase-amplitude-coupling (PAC). In mice, PAC deficits, which reflect cortical hyperexcitability, start long before symptom onset. Mechanistically, we unravel by mass spectrometry analyses of various CNS neuropeptides a selective and presymptomatic reduction of noradrenaline (NA) in the motor cortex of ALS mouse models, further validated by in vivo two-photon calcium imaging in behaving SOD-1G93A mice that reveals a concomitant massive reduction of locomotion-associated NA release. NA level deficits are also detected in post-mortem ALS patient tissues, along with transcriptomic alterations of the noradrenergic signalling pathway. Pharmacological intervention in mice demonstrates a critical role for NA in cortical hyperexcitability and PAC deficits in ALS. Our findings suggest that PAC could be a novel means to reveal cortical dysfunction in ALS, and unravel a critical role for neuromodulatory alterations in the pathophysiology of ALS, paving the way for hitherto non-explored therapeutic strategies.

96. Novel functionalized nanoparticles targeted to 18KDa translocator protein (TSPO) to track and modulate neuroinflammation in animal models of fALS

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Background. Neuroinflammation is recognized as a pathological hallmark and potential therapeutic target for many neurodegenerative diseases including Amyotrophic Lateral Sclerosis (ALS). However, neuroinflammatory responses are heterogeneous and reflect not only the extent of neuronal demise but also variable engagement glial cells in the attempt to cope with the neuronal damage. Thus, new pharmacological tools targeting specific cell subpopulations are warranted. We hypothesized that TSPO ligands, already widely used in the clinic to track neuroinflammation through PET, could be exploited to achieve selective cell targeting via a novel theranostic platform based on MRI/PET traceable nanoparticles (NPs).

Aims. In this work we aimed at: i) obtaining an in-depth analysis of TSPO distribution and correlation with disease stage in ALS animal models; ii) validating TSPO-targeted nanoparticles as potential novel cell-specific pharmacological tool.

Methods. We performed in-situ hybridization (ISH) and immunohistochemistry (IHC) experiments to investigate the expression and distribution of TSPO in the CNS of transgenic SOD1(G93A) rat model of ALS, which recapitulates the heterogeneous disease manifestations observed in patients. In parallel, we developed and validated novel polymeric NPs functionalized with TSPO-ligands.

Results, conclusions. We confirmed by ISH/IHC a clearcut upregulation of TSPO in microglia cells in the CNS areas most severely affected by the disease. Functionalization of NPs with two TSPO-selective PET tracers (PBR-28, PK11195) determined a TSPO-dependent NPs internalization in microglia cells both in vitro and in vivo. Based on these results, we launched a proof-of-concept preclinical study (in progress) to test the therapeutic potential of NPs targeting the NF-kB proinflammatory pathway.

97. Novel humanised conditional mouse models of SOD1-ALS

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Modelling late onset neurodegenerative disease is challenging, and improved models are needed to more faithfully recapitulate human pathology and develop treatment strategies. Towards this goal we have engineered three new mouse models of SOD1 Amyotrophic Lateral Sclerosis (SOD1-ALS) by genomic humanisation at the endogenous Sod1 locus (hSOD1-WT control mice), and by introducing disease associated mutations into the humanised allele (hSOD1-A4V and hSOD1-G93A mutant mice).

Humanisation of the mouse Sod1 gene was achieved in ES cells via CRISPR/Cas9- mediated homologous recombination with a targeting construct carrying the full length human SOD1 gene (13.9 kb knock-in). Our humanised SOD1-ALS models also carry a second conditional copy of exons 4 and 5 (designated exon 4' and 5'), allowing conditional expression/silencing of mutations introduced into exons 4 and 5 following Cre-Lox recombination. The hSOD1-G93A strain harbours a G93A point mutation in exon 4', enabling conditional expression of the SOD1-G93A mutant protein.

The knock-in mouse models express human SOD1 at physiological levels, which should avoid artefactual phenotypes arising from SOD1 overexpression, which is a drawback of transgenic mice. Here we present our work to characterise these novel humanised physiological mouse models of SOD1-ALS using a range of methods.

Humanised hSOD1-WT mice have undergone motor and molecular phenotyping up to 18 months of age with no ALS-like phenotype observed, as expected, indicating that human SOD1 can functionally replace mouse Sod1. Humanised hSOD1-G93A mice are currently undergoing allele quality control. Humanised hSOD1-A4V mice have undergone motor phenotyping up to 24 months of age, and are currently undergoing molecular phenotyping – with some mild ALS-like phenotypes noted.

We anticipate that such novel humanised mouse models will help to answer key questions surrounding early disease pathomechanisms in ALS. We also envisage these humanised models will serve as more accurate models to test future therapeutics, particularly antisense oligonucleotide (ASO) therapies, where the level of gene expression/knockdown is key. Furthermore, the conditional aspect of these strains will enable rescue experiments and studies involving tissue-specific expression of mutant SOD1.

98. Novel insights on the role and therapeutic potential of Glycoprotein nonmetastatic melanoma protein B (Gpnmb) in Amyotrophic Lateral Sclerosis.

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Background.

Increased levels of a peptide derived from Gpnmb in the cerebrospinal fluid (CSF) were recently associated with a poor prognosis in patients affected by Amyotrophic Lateral Sclerosis (ALS). On the other hand, other studies highlighted that upregulation of Gpnmb could play a neuroprotective and immunomodulatory role.

Objectives.

In this study we engaged an in-depth characterization of Gpnmb alterations in SOD1.G93A transgenic (TG) rat model of ALS and in patients, to clarify the value of Gpnmb as prognostic biomarker and to identify a precise time-window, during the disease process, suitable for successful therapeutic intervention.

Methods.

We applied in-situ hybridization (ISH) and immunohistochemistry (IHC) in the central and peripheral nervous system, coupled to the assessment of Gpnmb ectodomain (sGpnmb) in the CSF and blood of TG rats. In parallel, sGpnmb was assessed in a small cohort of ALS patients.

Results and discussion.

Gpnmb is mainly expressed in MNs in healthy conditions. However, in TG animals there is an early decrease of Gpnmb mRNA and protein levels in MNs and upregulation in reactive microglia after symptom onset. ISH and IHC highlighted a critical role for glial cells in the synthesis and release of sGpnmb. In parallel, we spotted a significant increase of sGpnmb in the CSF and blood of TG rats, as well as in ALS patients, when the pathology is more severe. After identifying the correct dose of Gpnmb for triggering the neuroprotective pathway activation in the rat model (proved by phosphorylation of AKT), we are currently running a preclinical proof of concept study to verify the therapeutic potential of early administration of recombinant Gpnmb while monitoring sGpnmb as biomarker of target engagement.

99. Novel nuclear bodies bridge FUS autoregulation and phase separation and are disrupted by ALS mutations

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Mutations in the FUS gene and resultant dysfunction of FUS protein have been linked to a subset of amyotrophic lateral sclerosis (ALS). FUS protein normally resides in the nucleus whereas ALS mutations lead to its mislocalisation to the cytoplasm of neurons, where it aggregates and forms inclusions. The precise mechanisms underlying the massive accumulation of mutant FUS in the cytoplasm are unclear, although secondary triggers, or second hits, e.g. persistent stress may be contributory. FUS protein is known to self-regulate its expression, through promoting retention of introns 6 and 7, whereas ALS-causing mutations were shown to disrupt this regulatory property. In the current study, we found that FUS transcripts with retained introns 6 and 7 are semi-extractable and form a novel phase-separated nuclear body we termed “FUS body”. FUS bodies form in human motor neurons and are significantly depleted in mutant FUS expressing cells including patient fibroblasts. Surprisingly, we found that normal FUS protein is dispensable for FUS body/intron retention regulation, suggesting that mutant FUS negatively affects FUS bodies via a gain-of-function mechanism. We also found that FUS bodies are stress-responsive and are disrupted by the majority of neurodegeneration-relevant stresses. Finally, we developed a cellular screening assay, based on high-content confocal imaging, using FUS body numbers as a readout, suitable for the identification of small molecule modulators of FUS alternative splicing. Using this assay, we screened a library of kinase inhibitors in WT cells and identified a compound – positive modulator of FUS bodies. This compound was also capable of restoring FUS body numbers in mutant FUS expressing cells. In conclusion, we refine the existing model of FUS autoregulation and provide evidence for a gain of function effect of mutant FUS on this mechanism. We also propose an approach to identify pharmacological agents for the manipulation of physiological homeostatic mechanisms controlling (mutant) FUS expression.

100. Novel transcriptional and mutational signatures in amyotrophic lateral sclerosis revealed by multiomics and machine-learning

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Despite the increasing understanding of the genetic components of ALS, their biological meanings are still poorly understood. Indeed, it is still not clear to which extent the pathological features associated with this disease are commonly shared across the heterogenous spectrum of cases. To answer this question, we performed transcriptional, epigenetic and mutational analysis of hiPSC-derived motor neurons carrying mutations in the C9orf72-, TARDBP-, SOD1- and FUS genes, as well as datasets from patients' biopsies. We identified a common signature, converging toward increased stress and synaptic abnormalities, which reflects a unifying transcriptional program in ALS. In addition, whole genome bisulfite sequencing linked the altered gene expression observed in mutant cells to their methylation profile, highlighting deep epigenetic alterations as part of the abnormal transcriptional signatures linked to ALS. We then applied multi-layer deep machine-learning to integrate blood and spinal cord transcriptomes and found a statistically significant correlation between their top predictor gene sets, which were significantly enriched in toll-like receptor signaling. Notably, the overrepresentation of this biological term also correlated with the transcriptional signature identified in mutant hiPSC-derived motor neurons, highlighting novel insights into ALS marker genes in a tissue-independent manner. Finally, using whole genome sequencing in combination with deep learning, we generated the first mutational signature for ALS and defined a specific genomic profile for this disease, which is significantly correlated to aging signatures, hinting at age as a major player in ALS. All in all, this work describes innovative methodological approaches for the identification of disease signatures through the combination of multi-omics analysis and provides novel knowledge on the pathological convergencies defining ALS.

101. Nucleocytoplasmic transport factors as modifiers of TDP-43 proteinopathy

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Cytoplasmic mislocalization and aggregation of TAR DNA-binding protein-43 (TDP-43) is a hallmark of the amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) disease spectrum. While most ALS cases are sporadic, mutations in TDP-43 can directly cause ALS, likely via a combination of nuclear loss of function and cytoplasmic toxic gain of function phenotypes.

Here we show that karyopherin beta-1 (KPNB1) and other members of the nuclear import receptor (NIR) protein family can rescue the hallmarks of TDP-43 proteinopathy, by restoring its solubility and nuclear localization, and reducing neurodegeneration in cellular and animal models of ALS/FTD.

Our findings suggest a novel NLS-independent mechanism where analogous to its canonical role in dissolving the diffusion barrier formed by phenylalanine and glycine-rich nucleoporins (FG-Nups) in the nuclear pore, KPNB1 is recruited into TDP-43/FG-Nup co-aggregates present in TDP-43 proteinopathies and therapeutically reverses their deleterious phase transition, mitigating neurodegeneration.

102. OMIC characterization of patient-derived spinal cord organoids to assess novel genes associated with C9orf72-Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron (MN) disease with adulthood onset and still represents a prominent health issue, as our knowledge regarding its pathogenesis is currently lacking. ALS pathophysiology involves progressive MNs degeneration in brain and spinal cord that leads to irreversible muscle atrophy and death within few years. In the context of multifactorial diseases like ALS, 3D models are a promising powerful tool that can recapitulate the complex architecture of tissues in a more accurate manner than 2D cultures. This work aims to characterize induced pluripotent stem cell-derived spinal cord organoids (SC-Orgs) and depict their ALS phenotype with a transcriptomic and proteomic approach, assessing novel ALS-associated genes/pathways. Our SC-Orgs displayed neural progenitors, post-mitotic neurons, MNs, and glial cells. In particular, the presence of proliferating neural cells gathered in stemness niches (SOX2+) decreased in time, whereas mature neuronal markers increased, indicating organoid maturation. SC-Orgs generated from 3 C9-ALS and 3 isogenic cell lines were collected at 30, 55, and 80 days in vitro (DIV) and evaluated for their morphology and neurodevelopmental features by immunohistochemistry, western blot, and qPCR. Specifically, DIV80 SC-Orgs expressed SMI32, TUBB3, MAP2, DCX, OLIG2, PAX6, HOXB4, and GFAP. Besides astrogliosis, the C9 condition interestingly showed Peripherin (PRPH) aggregation, as described in literature. Mass spectrometry and respective gene ontology highlighted significant dysregulation in cytoskeletal coordination, DNA damage response, ATP metabolic process, and RNA splicing and metabolism in C9-ALS cellular lines compared with their respective isogenic lines. Reactome FI network outlined enrichment in pathways related with ALS and other neurodegenerative diseases. Single-cell RNA sequencing followed by gene annotation disclosed the predominance of neuroectoderm and neural cell populations in the samples, remarking the potential of this disease model; in particular, C9-ALS SC-Orgs showed a reduced level of maturity, a condition observed by many researchers, despite ALS is not considered a neurodevelopmental disorder. Our omics data are currently under further examination, but we hope that our project might allow the assessment of novel candidate genes and proteins associated with C9ORF72-ALS pathogenesis and their potential as therapeutic targets.

103. Phenotype changes of microglia in SOD1G93A mice after mGluR5 genetic down regulation.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which several mechanisms concur to motoneuron (MN) damage. We demonstrated that partially deleting or ablating metabotropic glutamate receptor 5 (mGluR5) in the SOD1G93A ALS mouse model improved disease progression, increasing survival probability [1,2]. Moreover, the chronic pharmacological treatment of SOD1G93A mice by using CTEP, a mGluR5 negative allosteric modulator, positively affected ALS progression [3], thus supporting a central role of mGluR5 in driving ALS. Since ALS is a multicellular disease, where microglia play a pivotal role in MN damage, a better understanding of mGluR5-mediated modulation of microglia phenotype would be worthwhile for future cell-selective therapeutic interventions.

This study investigated the dynamic changes of SOD1G93A microglia phenotype induced by the mGluR5 genetic downregulation during disease progression.

Spinal cord microglia were acutely purified from WT and SOD1G93A mice of 30 (pre-symptomatic) and 120 (end-stage) days of life. The expression of pro- and anti-inflammatory markers was detected by flow cytometry. The bioenergetic profile and the redox balance were evaluated by oximetric, luminometric, and enzymatic assays.

Spinal cord microglia isolated from 120-day-old SOD1G93A mice displayed a distinct inflammatory pattern with respect to WT, the former showing higher expression of CD40, while the latter of CD206, CD163, and iNOS. Most relevant, microglia from 120-day-old SOD1G93A mice differed from that of 30-day-old SOD1G93A mice, showing higher MHCII, CD16-32, ARG1, and CD86 expression. Of note, the mGluR5 genetic deletion did not affect the inflammatory pattern of WT and SOD1G93A microglia at both ages. Biochemical studies highlighted increased oxygen consumption and decreased ATP synthesis, accompanied by increased anaerobic glycolysis in 30 and 120-day-old SOD1G93A mouse microglia versus age-matched WT. Moreover, the antioxidant responses and lipid peroxidation massively augmented in SOD1G93A microglia at both ages. Differently from the inflammatory phenotype, the mGluR5 genetic deletion restored the bioenergetic and oxidative alterations observed in SOD1G93A microglia.

Our data demonstrated that the mGluR5 genetic deletion did not affect the inflammatory status of SOD1G93A spinal cord microglia, but normalized the energetic metabolism failure and the altered redox status.

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104. Phosphorylated Tyr526 FUS in FTD

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Fused in Sarcoma (FUS), a normally nuclear DNA/RNA-binding protein, forms abnormal cytoplasmic inclusions in the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD). In ALS, these inclusions are associated with mutant FUS, whereas in FTD they consist of mutation-free FUS. As Tyr526 phosphorylation inhibits nuclear import of FUS, our aim in this study was to determine the extent of Tyr526-FUS phosphorylation in vitro and in vivo in mouse brain and human postmortem brain tissue. Tyr526 phosphorylation of FUS by Src-family kinases indicated impaired nucleocytoplasmic distribution and aggregation of FUS in cell models, and pronounced phospho-Tyr526 co-localization with pSrc/pAbl was detected in mouse brain. Brain region-specific phospho-Tyr526 FUS co-localization with active pSrc/pAbl kinases in mice pointed to preferential involvement of cAbl in cytoplasmic phospho-Tyr526 FUS mislocalization in cortical neurons. Final analysis of the detail patterns of active cAbl kinase and phospho-Tyr526 FUS in the neurons of human post-mortem frontal cortex brain tissue demonstrated altered cytoplasmic phospho-Tyr526 FUS distribution in the cortical neurons of FTD patients as compared to control. Considering the overlapping patterns of cAbl activity and phospho-Tyr526 FUS distribution in cortical neurons, we propose that cAbl kinase is involved in mediating cytoplasmic toxic FUS mislocalization in FTD and patients, likely leading to differences in disease progression.

105. Presence of stathmin-2 cryptic exon alone is not sufficient to determine clinical phenotype in amyotrophic lateral sclerosis/frontotemporal dementia

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TDP-43 mislocalisation and accumulation in pathologically phosphorylated cytoplasmic aggregates is a hallmark of amyotrophic lateral sclerosis frontotemporal dementia spectrum disorders (ALSFTSD). The presence of TDP-43 aggregation is a specific marker of regional phenotypic presentation in these diseases, however, lacks sensitivity as a proportion of ALSFTSD cases has a substantial burden of TDP-43 pathology but no associated clinical manifestations. The recent identification of cryptic exons, as molecular markers of TDP-43 loss-of-function, are thought to be a more sensitive signature of TDP-43 pathology and thus clinical phenotype. Here we set out to understand the clinico-pathological association between the presence of the STMN-2 cryptic exon (CE) and clinical phenotype, by designing and validating BaseScope in situ hybridisation probes that specifically bind to incorrectly spliced STMN-2(CE) and to normally spliced STMN-2(N). We assessed expression of these two transcripts in deeply phenotyped post-mortem tissue from a cohort of ALSFTSD patients with diverse pathological and clinical phenotypes. We show that the STMN-2(CE) is seen in ALS cases exhibiting TDP-43 pathology with an associated cognitive phenotype. However, STMN-2(CE) was also seen in a control case with age-related nuclear phosphorylated TDP-43 pathology, and there was minimal STMN-2(CE) expression in a cognitively affected ALS case with abundant TDP-43 pathology, possibly due to reduced stability. However, despite this variation in cryptic exon detection, phenotypically affected cases always showed a reduction in STMN-2(N) expression. We propose that a ratio of STMN-2(N) to STMN-2(CE) expression may provide additional sensitivity and specificity, compared to STMN-2(CE) expression alone, and therefore may be more clinically informative in accurately determining clinical phenotype.

106. Pridopidine exerts neuroprotective effects via activation of the Sigma-1 receptor (S1R)

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Pridopidine is a small molecule with selective and robust sigma-1 receptor (S1R) activity. The S1R is a compelling therapeutic target as S1R mutations cause juvenile and adult ALS. The S1R localizes to the mitochondrial-associated ER membrane (MAM) and maintains MAM integrity. Disruptions in MAM integrity are associated with ALS and induce cellular pathologies including altered neurite outgrowth, decreased autophagy, impaired synaptic function, decreased BDNF transport, and ultimately, neurodegeneration. Pridopidine provides neuroprotection by restoring MAM integrity and improving downstream pathways.

Key features of ALS are ameliorated by pridopidine in SOD1G93A mice. Pridopidine increases body weight and muscle fiber, reduces mutant SOD1 aggregates, and improves motor coordination of SOD1G93A mice. SOD1G93A motor neurons have improved synaptic function, BDNF transport, and survival with pridopidine treatment. Here, we further investigate pridopidine's mechanism of action (MoA) through its regulation of neurite outgrowth and autophagy.

Neurite outgrowth is essential for maintenance and repair of motor neuron axons and is disrupted in ALS models. Primary motor neurons from hTDP-43ΔNLS mice have reduced neurite outgrowth compared to control (~35%, $p<0.0001$). Pridopidine increases neurite outgrowth of hTDP-43ΔNLS motor neurons (~25%, $p=0.0012$).

In the ALS-causative C9orf72 mutation, G4C2 RNA expansions disrupt nucleocytoplasmic transport (NCT) of transcription factor EB (TFEB), a regulator of autophagy. NSC34 cells expressing (G4C2)31 repeats have reduced NCT of TFEB (~40%, $p<0.05$) and autophagy (~30%, $p<0.01$). Pridopidine rescues NCT of TFEB (~90%, $p<0.01$), increases autophagy (~50%, $p<0.05$), and provides neuroprotection (~12%, $p<0.001$).

These results support pridopidine's MoA, providing robust neuroprotection through activation of the S1R. Pridopidine was recently evaluated in the Phase 2 HEALEY ALS Platform Trial. While the primary endpoint of change in ALSFRS-R at 24 weeks was not met, beneficial effects were observed across several secondary and exploratory endpoints. Both pre-specified and post-hoc analyses in early, fast progressors showed benefits in speech, ALSFRS-R total, respiratory and bulbar subdomains as well as in quality-of-life measures and neurofilament light (NfL); results to be presented by the Healey Center for ALS. These findings support the therapeutic potential of pridopidine in ALS and inform design of a planned phase 3 trial.

107. Propagation of SOD1 pathologic determinants in human iPSC-derived motor neurons.

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Amyotrophic Lateral Sclerosis (ALS) is a disorder leading progressively to the complete paralysis of patients due to degeneration of motor neurons (MNs). As disease onset is often anatomically localized, a hypothesis is that the disease could spread to contiguous regions from cell to cell. One major pathological hallmark observed in patient's tissues are accumulations of misfolded proteins, such as TDP-43 or SOD1. We propose that accumulations of these proteins could lead progressively to cell overload and saturation of conventional degradation pathways and be responsible for the activation of the unconventional secretory pathway, dependent on the deubiquitinase USP19, called the Misfolded Associated Protein Secretion (MAPS) pathway. This pathway removes ubiquitin residues from misfolded proteins, transfer these proteins to DNAJC5 chaperones, then into late endosomes, and release them into the extracellular space with a possible uptake by surrounding cells. This specific pathway was particularly studied for secretion of a-synuclein or Tau, but studies were conducted with plasmid over-expressing cellular models. To determine whether the USP19-dependent secretion of the SOD1 pathogenic determinant occurs in ALS affected cells, we used MNs derived from induced pluripotent stem cells (iPSC) of 4 patients carrying mutations in SOD1 as well as isogenic or non-related controls. Our results reveal that misfolded SOD1 proteins stained with C4F6 anti-misfolded SOD1 antibodies accumulate in MNs carrying SOD1 mutations and that these accumulations colocalized with the DNAJC5 chaperone protein. Interestingly we observed SOD1 secretion within less than 2 hours in MN media. To assess whether SOD1 secretion is USP19-dependent, we have designed lentiviral vectors allowing the overexpression or the downregulation (using siRNA) of USP19 and we will compare their effects on SOD1 secretion. Trans-well cultures will be established to investigate the possible USP19-dependent propagation of SOD1 from MN to MN. Moreover, we are also investigating whether the MAPS pathway could also be involved in the secretion and propagation of misfolded TDP-43. In conclusion, this study could allow the identification of pathways involved in the propagation of pathological determinants, pathways that could be of interest to block ALS progression.

108. Propranolol reduces the accumulation of cytotoxic aggregates in C9orf72-ALS/FTD in vitro models

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Mutations in the C9orf72 gene are the most common cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The pathogenetic mechanisms linked to this gene are a direct consequence of an aberrant intronic expansion of a GGGGCC hexanucleotide located between the 1a and 1b non-coding exons, which can be transcribed to form cytotoxic RNA foci or even translated into aggregation-prone dipeptide repeat proteins. Remarkably, the repeat expansion also affects the expression levels of C9orf72 itself, suggesting haploinsufficiency as a pathomechanistic contributor. Thus, disease pathogenesis in C9orf72 mutations appears to be driven by a double-hit mechanism combining loss of function and toxic gain of function.

In this study, we aimed at identifying a strategy to address both aspects of the C9orf72-related pathobiochemistry and provide proof-of-principle information for a better understanding of the mechanisms leading to neuronal loss. By using primary neurons overexpressing toxic poly(GA), the most abundant protein product of the GGGGCC repeats, we found that the antiarrhythmic drug propranolol could efficiently reduce the accumulation of aberrant aggregates and increase the survival of C9orf72-related cultures. Interestingly, the improved catabolism appeared to not depend on major degradative pathways such as autophagy and the proteasome. By analyzing the proteome of poly(GA)-expressing neurons after exposure to propranolol, we found that the drug increased lysosomal degradation through a mechanism directly involving C9orf72 protein, whose levels were increased after treatment. Further confirmation of the beneficial effect of the beta blocker on aggregates‘ accumulation and survival of hiPSC-derived C9orf72-mutant motoneurons strengthened the finding that addressing both facets of C9orf72 pathology might represent a valid treatment strategy. Moreover, the effects of propranolol on lysosomal degradation open up a new perspective on how to therapeutically target autophagy blockage in these ALS/FTD cases.

109. Proteomic Changes following Terazosin Treatment in a TDP-43 Mouse Model

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Energy metabolism is known to be dysregulated in ALS regardless of genetic background, so targeting ATP production could be a therapeutic strategy applicable across patient groups. Terazosin is a repurposed drug that was found to have a non-canonical action of increasing PGK1 activity, a glycolysis enzyme. Treatment with terazosin has been shown to be neuroprotective in other diseases such as Parkinson's disease, and we have shown a therapeutic effect in multiple models of ALS. We examined proteomic changes in the spinal cord of homozygous Thy1-hTDP-43 overexpression mice compared to subclinical heterozygous and non-transgenic controls to analyse disease-associated pathways, as well as assessing the impact of terazosin treatment to determine mechanisms of neuroprotection. Thy1-hTDP-43 mice and littermate controls were treated daily, intraperitoneally with 100 µg/kg terazosin or saline control until late-symptomatic stage (P19), when lumbar spinal cord was collected. Protein from 5 mice per group was collected and pooled for TMT-mass spectrometry analysis. Peptide identifications were utilised to generate protein identities using MASCOT search engine, while those identified by >2 unique peptides were taken forward for analysis. Expression changes in heterozygous and homozygous Thy1-hTDP-43 mice were compared to wild-type, and terazosin-treated Thy1-hTDP-43 mice were compared to saline control. Protein expression changes were assessed using Ingenuity Pathway Analysis and BioLayout Express3D. To determine the effect of TDP-43 overexpression in the spinal cord, homozygous Thy1-hTDP-43 mice were compared with heterozygous and non-transgenic controls. 4 unique clusters distinguishing expression patterns between groups were generated. The cluster showing mild upregulation in heterozygous mice and comparably further upregulation in homozygous mice contained proteins associated with ribosomal processing, ubiquitin conjugation and mRNA processing, while the cluster showing a trend in downregulation across genotypes contained proteins associated with mitochondria, oxidative phosphorylation and protein folding. Comparison between homozygous Thy1-hTDP-43 mice treated with terazosin and saline control shows an activation of oxidative phosphorylation and an inhibition of mitochondrial dysfunction pathways. These results suggest that this TDP-43 mouse model has dysregulated energy metabolism in the spinal cord, and that this can be targeted therapeutically by terazosin.

110. Regulation of stress granules' formation with plant extracts containing polyphenols

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Polyphenols are a diverse group of naturally occurring compounds found in plants, which have garnered significant attention in recent years due to their potential health benefits. Accumulating evidence suggests that polyphenols possess various biological activities, including antioxidant, anti-inflammatory, and neuroprotective properties. Neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS), represent a significant global health burden. One area of interest in the context of neurodegenerative diseases is the role of stress granules (SGs) in cellular stress response and their contribution to disease pathology. SGs are dynamic, cytoplasmic aggregates of RNA and proteins that form in response to various stressors and function as a protective mechanism to facilitate cellular recovery. However, aberrant SG dynamics and persistence have been implicated in the development and progression of neurodegenerative diseases, including ALS. The potential of polyphenols as therapeutic agents for neurodegenerative disorders, particularly in the context of SG formation and regulation have been explored. For instance, resveratrol and other polyphenols, such as epigallocatechin gallate and curcumin, have been demonstrated to influence SG formation, reduce oxidative stress, and mitigate neuroinflammation in models of ALS and other neurodegenerative diseases. Our aim was to explore the effects of plant extracts not a single polyphenol on SG formation and neurodegeneration. We prepared extracts from 3 knotweed species: Reynoutria sachalinensis, Reynoutria japonica) and Reynoutria × bohemica to test their antioxidant activity on our cell model SH-SY5Y Flp In mScarlet-G3BP1-myc, which expresses the stress granule protein G3BP1 fused with mScarlet. None of the knotweed extracts containing polyphenols were cytotoxic, moreover, they proved to be potentially neuroprotective. When we exposed cells to extracts of giant knotweed, Japanese knotweed, or Bohemian knotweed and induced oxidative stress with sodium arsenite, the cells contained less SG compared to the control indicating that antioxidant activity of the extracts regulate SG dynamics. Further studies on molecular mechanism underlying plant extracts'-mediated neuroprotection could give new opportunities for the development of extract-based therapeutic interventions for ALS and other neurodegenerative diseases.

111. Reprogrammed astrocytes from a C9-ALS family with variable penetrance display differential C9orf72 pathology and motor neuron toxicity in co-culture

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Hexanucleotide repeat expansions in C9orf72 (C9-HRE) are the most common genetic risk factor for amyotrophic lateral sclerosis (ALS). The triggers for the switch between the asymptomatic and symptomatic disease state, which emerges with age, are poorly understood. Here we describe a C9-HRE+ family with an asymptomatic father and discordant identical twins. The affected twin presented with ALS at age 35, with a biopsy collected two years into disease, and a second, four years later, at end-stage (86 months disease duration). This individual was a high-intensity athlete prior to onset of symptoms, associated with the development of ALS in C9-HRE Carriers (Julian et al, 2021). The unaffected twin had a comparatively sedentary lifestyle.

We generated induced neural progenitor cells (iNPC) and derived astrocytes (iNPC-As) from the fibroblasts of these individuals to establish if we could replicate the family's variable penetrance, understand the pathological features, and discern if pathology correlated with disease progression.

Fibroblasts were directly converted into iNPCs as previously described (Meyer et al,. 2014). C9orf72 pathology was assessed using fluorescent in situ hybridisation for RNA foci and MSD ELISA for dipeptide repeat proteins (DPR). Toxicity was determined by survival after 72-hour co-culture with murine HB9-GFP motor neurons.

We first evaluated C9-HRE length by Oxford Nanopore sequencing, the father carrying 70 repeats and 800 in both twins. Whole genome sequencing did not detect any significant gene variant that could cause the differential clinical status of the twins. We next proceeded to assess the presence of RNA foci. While in iNPC-As we could not detect antisense foci, nuclear sense foci were observed in 37% of the affected twin's cells at symptom-onset (HC vs Af. Early P = <0.0001), but only 1% at end-stage, 3% of unaffected twin's cells and <1% in the father. Interestingly, only the affected twin at symptom-onset had significant production of poly-GP and poly-GA DPRs, ablated in end-stage astrocytes (HC vs Af. Early P = <0.0001). Interestingly, when assessed in co-culture, the iNPC-As derived from the end-stage biopsy were significantly more toxic to MNs than the iNPC-As derived from the early disease stages (HC vs Af. Late P = 0.0036), thus indicating that this model might be recapitulating important aspects of disease progression. We plan to shed light on such mechanisms by performing RNA-sequencing in these astrocytes

112. Resistant and vulnerable motor neurons show unique temporal gene regulation in SOD1G93A ALS

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Differential vulnerability in SOD1G93A mice has been characterized phenotypically but the longitudinal transcriptional responses of resistant and vulnerable motor neuron populations remain elusive. Thus, we have conducted RNAseq of resilient somatic oculomotor and trochlear (CN3/4) motor neurons, as well as visceral vagus nerve (CN10) motor neurons and compared to vulnerable hypoglossal (CN12) and spinal motor neurons (sMNs) isolated at presymptomatic (P56) and symptomatic (P112) stages. Differential gene expression analysis showed that each neuron group and disease stage show a distinct gene regulation with only a minority of genes being regulated across ages and cell types. Some of the disease DEGs in CN3/4 and CN10 had an opposite and significant expression in vulnerable neurons at symptomatic age. We believe these DEGs may lie at the heart of their differential ability to adapt to disease. As sMNs displayed the largest number of DEGs with disease, we used three generations of pathway enrichment methods (over-representation, per-gene and network enrichment analysis) to analyze these. Our results revealed deregulated pathways related to neuropeptide signaling, PERK-mediated unfolded protein response, ER stress and metabolic processes. We subsequently cross compared our data with published transcriptomics sMN data sets to identify robust vulnerability signatures across SOD1 mutations. This revealed that PERK-mediated unfolded protein response and positive response to ER stress pathways were common to SOD1G93A and SOD1G37R mutations. We also used two classification methods (random forest and support vector machine) on a published single cell RNAseq dataset with the SOD1E1OOG mutation. This analysis suggests that a subset of the DEGs we found dysregulated in SOD1G93A sMNs are general markers of SOD1-ALS. In conclusion, our analysis shows that each motor neuron subpopulation responds uniquely to the SOD1G93A mutation, and that different disease stages give rise to distinct transcriptional responses. Cellular stress pathways were consistently enriched in sMNs across SOD1 mutations and data sets and could explain the unique and specific vulnerability of sMNs.

113. RIPK1 activation contributes to ALS pathophysiology and drives a pro-inflammatory gene signature in a human iPSC-derived tri-culture system

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Background: Receptor-interacting serine/threonine protein kinase 1 (RIPK1) is a critical regulator of inflammatory signaling and cell death and is implicated in pathogenic cellular pathways of amyotrophic lateral sclerosis (ALS). Utilizing the SOD1G93A mouse model of ALS, we have previously shown an increase in RIPK1 activation and expression in the spinal cord, with delayed symptom onset and motor impairment upon RIPK1 inhibition. Further, RIPK1 activation and expression were elevated in post-mortem spinal cord samples from sporadic ALS patients.

Objective: While these data suggest activation of RIPK1 in the central nervous system (CNS) may mediate ALS disease progression, we sought to interrogate RIPK1-induced gene expression changes in an in vitro tri-culture system and elucidate the role of RIPK1 activity in ALS patients by correlating gene changes with RIPK1 expression using single nucleus RNA-sequencing.

Methods: We established a human induced pluripotent stem cell (iPSC)-derived tri-culture system comprising of motor neurons, microglia, and astrocytes to derive a human RIPK1-dependent gene signature. Human ALS post-mortem spinal cord samples were obtained from the NIH NeuroBioBank. RIPK1 activation and expression levels in the samples were assessed by Meso Scale Discovery (MSD) assay. In vitro and in vivo RIPK1-dependent gene and pathway regulation were assessed using single cell and single nucleus RNA-sequencing, respectively.

Results: We identified an immune and neuro-inflammatory gene signature driven by microglia and astrocytes upon RIPK1 activation in the iPSC-derived tri-culture system. In post-mortem ALS spinal cord samples, we identified disease-associated microglia and astrocyte subpopulations and profiled the pathway changes in ALS patients. Increased RIPK1 expression may correlate with gene and pathway modulation and cell population shifts, namely loss of neurons and endothelial cells and increases in astrocytes and microglia, relative to control as assessed via single nucleus RNA-sequencing.

Discussion/Conclusions: Human data sets are being further explored to correlate transcriptomic changes with RIPK1 expression levels and understand pathway changes in ALS-associated cell type-specific subpopulations. Taken together, these data suggest that aberrant RIPK1 activation in various CNS cell types may contribute to motor neuron loss in ALS.

114. Role of MOK kinase in microglial TBK1/type-1 IFN pathway and mitochondrial function

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Neuroinflammation is a key pathological hallmark in amyotrophic lateral sclerosis (ALS) and a link has been established between microglial responses, particularly inflammatory and activated type-I IFN pathways, and the onset and progression of disease. In the last few years, TANK-binding kinase 1 (TBK1) -genetically associated to familial ALS cases- has emerged as a key inducer of type-I interferons and a major player in autophagy and mitophagy. Recently, we uncovered MOK as a Ser/Thr kinase that regulates inflammatory and IFNbeta/alpha responses in microglia and is involved in ALS. In this work, we explored the possible role and mechanisms of MOK in the TBK1/type-I IFN axis and mitochondrial function. By using MOK-KO microglial cells and stimulation with LPS, we found that MOK positively modulates phospho-TBK1 and phospho-p38 levels under inflammatory conditions as well as transcription levels of key interferon-stimulated genes (ISGs). Moreover, our data indicate that MOK has an impact on LPS-caused alterations in autophagy as measured by p62 and LC3 changes. Remarkably, our results also showed that MOK-KO microglial cells compromise mitochondrial function, according to Seahorse assessment. Collectively, our study points to a novel upstream regulator of TBK1/type-I IFN/mitochondrial events in neuroinflammation.

115. Simultaneous activation of two complementary targets, Kv7.2/3 and TSPO: a promising and novel treatment for Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of motoneurons (MNs) in the motor cortex and spinal cord, leading to muscle weakness and atrophy. Currently, there is no effective therapy for ALS and patients die within 2-5 years after the disease onset. Given ALS is a multifactorial disease in which a wide variety of pathophysiological mechanisms have been reported to contribute to MN degeneration, targeting several of these altered mechanisms could result in well-suited ALS treatment.

In this project, we evaluate whether the simultaneous activation of two complementary targets, the voltage-gated potassium channels 7.2/3 (Kv7.2/3) and the mitochondrial translocator protein TSPO, by the novel synthesized GRT-X chemical compound could be an effective neuroprotective treatment in vitro and in vivo MN degenerative models.

Data showed that GRT-X increases MN survival in spinal cord organotypic cultures (SCOCs) under toxicity induced by astrocyte conditioned mediums (AMC) harvested from i) hSOD1G93A transgenic mice astrocytes ii) astrocytes differentiated from human iPSCs from SOD1D90A and TDP43A90V ALS patients. In vivo, daily oral administration of GRT-X compound in SOD1G93A mice preserves neuromuscular function and improves coordination and motor activity determined by electrophysiology, rotarod and treadmill tests. Histological studies proved that GRT-X promotes MN survival and neuromuscular junction innervation in treated SOD1G93A mice. These data highlight the effectiveness of the GRT-X compound for the treatment of ALS pathology.

116. Single cell RNA sequencing in isogenic FUS and TARDBP mutant ALS lines reveals early mitochondrial dysfunction as a common pathway in motor neurons

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Mutations in the RNA/DNA-binding proteins FUS and TDP-43 cause amyotrophic lateral sclerosis (ALS) with distinct neuropathological features. It is currently unclear how these gene mutations lead to selective motor neuron death and if there are common mechanisms across disease causations. Using single cell RNA sequencing of neurons derived from isogenic induced pluripotent stem cell lines, we show that motor neurons harbouring FUS P525L or FUS R495X mutations show a 4.9- to 15.5-fold larger transcriptional response than interneurons. About 20% (737 DEGs) of transcripts were coregulated across FUS R495X and P525L motor neurons and by comparing to a FUS knockout line we could discern that 48% (355 DEGs) were part of gain-of-function of FUS. Cross-comparing with isogenic TDP-43 M337V motor neurons, identified common mitochondrial dysfunction across FUS gain-of-function and TARDBP gene mutations, as did comparison with published RNA-Seq data from C9orf72-ALS motor neurons. Metabolic assessment confirmed a decrease in mitochondrial respiration and ATP turnover in mutant FUS and TARDBP lines and live cell microscopy showed a decrease in mitochondrial motility across ALS motor axons. Thus, we have identified early mitochondrial dysfunction in motor neurons shared across ALS-causative mutations, that could have major implications for their survival. Altogether our data furthers the understanding of the motor neuron-intrinsic roles of FUS and TARDBP in health and ALS and we identify targets that could be modulated therapeutically.

117. Single-cell RNAseq in Drosophila models of ALS to decipher non-cell autonomous mechanisms of neurodegeneration

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that rapidly affects motor neurons (MNs) in the brain and spinal cord. This disease, up to date, has no cure. In about 90% of cases the origin of the disease is unknown, but in 10% it is caused by Mendelian inherited genetic mutations in more than 20 genes, among which TARDBP, which encodes TDP-43 protein, and FUS stand out. Recent findings have contributed to question the neurogenic origin of the disease, by claiming that the supporting cells of the motor neurons within the neuromuscular junction (NMJ), such as glia cells and myoblasts from skeletal muscle, contribute to MN toxicity. The aim of this study is to analyze how the ALS-associated pathological inputs from different components of the NMJ impinge on MN transcriptome, to identify early changes to intervene. For this purpose, we have generated Drosophila melanogaster models that specifically silence or express TDP-43 or FUS in the tissues that are part of the NMJ: muscle, motor neuron and glia cells. Using single cell RNA sequencing technology, we analyze the transcriptomic profile of ventral nerve cord and brain MNs from these Drosophila models. In this research, we show the process and methodology of obtaining these samples, as well as preliminary results obtained in this study, whose final objective is to identify potential molecular targets on which to act to treat the disease.

118. SOD1-G93A mutation modifies cellular properties of mesenchymal stem cells in a mouse model of Amyotrophic Lateral Sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with, to date, no effective treatment. Mesenchymal stem cell therapy was proposed and has been tested in the past years as a potential therapy for ALS due to his neuroprotective effects mediated by trophic support and modulation of the immune response. Whereas the safety of its use has been widely demonstrated, the efficacy of these treatments is still controversial with inconsistent results. In this work, we studied if SOD1-G93A mutation modifies cellular properties of mesenchymal stem cells in a mouse model of Amyotrophic Lateral Sclerosis.

Methods: For this purpose, we characterized Bone Marrow (BM-) and Adipose derived (Ad-) Mesenchymal Stem Cells (MSCs) isolated from the ALS murine model SOD1G93A and compared them with MSCs isolated from their wild-type littermates. In order to determine in vitro proliferation, multipotency and trans-differentiation capacity, the following tests were performed: morphology, cell proliferation, characterization and neuronal trans-differentiation. Subsequently, the expression of genes involved in pluripotency, neuroprotection and immunoregulation was assessed by qPCR.

Results: We observed impairment in the proliferative abilities of Ad-MSCs from

transgenic mice, while differentiation potential was not altered. In general, WT-MSC cultures expressed higher levels of the pluripotency capacity markers. Specifically, it showed increased expression of c-Myc, Nanog and Oct4. GDNF, NT4, IGF-1(neutrophic factors) and TGF-β and IL-10 (genes involved in immune response and inflammation) were found to have a higher expression in wild-type over mutant cultures, especially in WT-BM-MSC.

Conclusions: Our results show differences in the properties of MSCs and BM-MSCs between the cultures of SOD1G93A model and wild-type mice that could be affecting different biological functions and be related to the mechanisms of the disease. These results are in line with our previous data on deficient function of muscle-derived stem cells. Thus, taken together, these data highlight the need for therapies in ALS to target, not only differentiated cells but also but also their stem cells.

119. Strain-specific propagation of templated SOD1 aggregation along neuroanatomical tracts in ALS mice

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Background and aim: Increasing evidence suggest that ALS and other neurodegenerative disorders share a common pathogenic mechanism involving a prion-like spread. In ALS, we have previously identified two distinct human SOD1 (hSOD1) aggregate strains, strain A and B. End-stage hSOD1G85R Tg mice display a typical strain A pattern while end-stage hSOD1D90A Tg mice display a mixture of strain A and strain B patterns. The younger the end-stage mice, the more of the latter. Strain A and B seeds transmit template-directed aggregation and premature fatal paralysis when inoculated into the spinal cord of asymptomatic hSOD1 Tg mice with mutations.

Our aim was to study the strain-specific spread of strain A or B hSOD1 aggregates following sciatic nerve injections. The sciatic nerve injection provides a model for investigating the spatiotemporal spread of hSOD1 strains along neuroanatomically defined pathways.

Methods: We prepared seeds from end-stage hSOD1G85R, hSOD1D90A Tg mice, and non-Tg control mice and inoculated them into the sciatic nerve of adult hSOD1G85R Tg mice. We used binary epitope mapping assay with strain-specific anti-hSOD1 antibodies to investigate templated aggregate conformations in the inoculated Tg mice. We also performed an immunofluorescence analysis of tissue sections from the CNS from different time points.

Results: We found that sciatic nerve inoculation of strain A and B seeds induced premature ALS in Tg mice and disease progression was slower in strain B-inoculated mice. Furthermore, strain B-inoculated mice with short lifespans showed ~ 100% strain B, whereas mice with intermediate to long lifespans showed mixed strain A and B aggregate structures. Aggregation pathology began in the lumbar spinal cord and progressed rostrally to several brainstem nuclei along descending motor tracts. Strain A and B aggregates had distinct morphology. While strain A aggregates displayed amorphous grain-like aggregates, strain B aggregates displayed fibrillar elongated aggregates.

Conclusions: Our results demonstrate prion-like properties of strain A and B seeds and the ability to spread hSOD1 aggregation pathology through retrograde axonal transport along neuroanatomical connections following sciatic nerve inoculations. Strain A and B showed different characteristics and differed in structure, progression rates, end-stage aggregate levels and histopathology. We have established a robust model of the progressive spread of hSOD1 aggregation pathology.

120. Structure and function of the C9ORF72-SMCR8-WDR41 complex and its implication in Amyotrophic lateral Sclerosis (

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Amyotrophic lateral sclerosis (ALS), the 3rd most common neurodegenerative disease, is characterized by degeneration of both upper and spinal motor neurons, resulting in skeletal muscle paralysis and death from respiratory failure generally in 3 to 5 years. The main genetic cause of ALS is an expansion of GGGGCC repeats in the C9ORF72 gene. These repeats promote DNA epigenetic changes that silence C9ORF72 expression. However, very little is known on the molecular and cellular roles of this protein.

We and other found that C9ORF72 interacts with the WDR41 and SMCR8 proteins to form a stable protein complex (Sellier et al., 2016). To better understand the molecular functions of C9ORF72, I solved its 3D structure by cryo-EM. While we successfully produced, purified and imaged the C9ORF72 complex, we noted that a large part of SMCR8 was not resolved due to conformational flexibility. Thus, we hypothesized that some protein partners are required to stabilize the C9ORF72 complex. To identify these interactants, we immunoprecipitated C9ORF72 and through mass spectrometry analyses, we found a novel interaction with ARL14, a small GTPase of ill-defined function. In addition, I observed by super-resolution microscopy that the C9ORF72 protein re-localizes to lysosomes upon mTOR and autophagy activation. Importantly, I also found a role of C9ORF72 in Autophagic Lysosome Reformation (ALR), a novel mechanism explaining regeneration and biogenesis of new lysosomes after mTOR and autophagy activation. This is highly relevant as elucidating the molecular mechanisms underlying ALS will open novel therapeutic route for this devastating disease.

Overall, my work unveils a novel partner (ARL14) to the C9ORF72 complex and an exciting novel function for this complex in lysosome biogenesis. This is important as dysfunctions of lysosomes may contribute to neuronal cell death in ALS. However, it remains to finalize this work and determine the cryoEM structure of the C9ORF72 complex with ARL14, as well as the importance of ARL14 in lysosome dysfunctions in ALS.

121. TDP-43 dependent splicing missregulation in ALS

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Although ~15% of ALS cases are inherited via identified genetic variation, ~85% of cases have unknown genetic origins. Therefore, drug development for ALS urgently needs to identify disease mechanisms representing the majority of ALS cases across diverse genetic backgrounds. Most (~98%) ALS cases present cytoplasmic mislocalization and aggregation of TDP-43 protein. This protein has several roles in RNA metabolism, including splicing regulation. Lost splicing regulation, following TDP-43's mislocalisation, can introduce undesired changes to target RNAs that impacts abundance and function of encoded protein products.

In this project we interrogated the functional target networks of TDP-43 different disease model systems (e.g. PM tissue, iPSC, immortalised cells). RNA-seq was used to identify mis-processed events, whilst complementary TDP-43 CLIP-seq data was used to identify TDP-43 binding found in close proximity. We have subsequently functionally validated certain events which we hypothesise could have relevance to ALS neural cell biology. Specifically, our experiments in cultured cells verified TDP-43 dependency of our targets by increasing the mis-spliced isoforms, reducing normal isoform abundance, and reducing the corresponding proteins. Cells accordingly show defects in cellular pathways regulated by our targets, and which are known to be disturbed in ALS. Finally, we also observed an increase of these same mis-spliced isoforms in hiPSC-derived MN (human induced Pluripotent Stem Cells-derived Motor Neurons) from ALS cases, and in ALS post-mortem tissue.

Together our results suggest a model in which lost regulation by TDP-43 introduces splicing defects in specific targets, that this reduces levels of their respective proteins, and that this ultimately compromises their regulated cellular pathways. We hypothesise that correcting these events could be neuroprotective for the majority of ALS cases displaying TDP-43 pathology.

122. TDP-43 mislocalisation as a normal stress response that goes awry in ALS

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Extensive loss of TDP-43 in motor neurons is a nearly ubiquitous feature of ALS. TDP-43 depletion and dysfunction were reported to cause aberrant splicing, in particular cryptic exon inclusion, the best studied example being alternative exon inclusion in Stathmin-2 pre-mRNA leading to truncated mRNA production and protein downregulation. A recent study indicated wide-spread translation of novel splice variants in cells affected by TDP-43 loss of function and de novo protein isoform generation. TDP-43 is a shuttling protein and its relocation to cytoplasm, where it can be recruited to stress granules, has been reported in response to different intrinsic and extrinsic stresses. Therefore, a partial reversible relocation of TDP-43 from the nucleus may be required during stress for the (neuronal) cell to mount efficient stress response. Temporary nuclear decrease of TDP-43, as a known negative regulator of paraspeckles (stress-responsive nuclear granules), may be critical for paraspeckle-regulated stress signalling. Yet, normal physiological function of acute, stress-inducible TDP-43 loss of function in healthy cells/neurons remains unstudied.

We hypothesise that transient TDP-43 depletion is an important physiological response to certain types of cellular stress and that novel protein variants produced in cells with transient TDP-43 loss of function mediate cytoprotective stress responses and have a physiological function. We have tested a panel of chemical and physical stressors, many of which induce SGs, for their ability to alter TDP-43 localisation in human motor neurons. The timeliness of TDP-43 dynamic changes have also been characterised. Stresses were classified for their effect on nuclear and cytoplasmic TDP-43 levels and their ability to induce nuclear and cytoplasmic granules. A panel of de novo protein (cryptic exon, CE) variants typical for TDP-43 depleted cells based on the recent proteogenomic study have been profiled using immunostaining and western blotting. Levels of respective cryptic RNA variants were quantified by qRT-PCR. Proteins with confirmed expression and localisation changes upon TDP-43 altering stresses have been shortlisted for proteomic validation of their proteomic networks and functional studies. This project has been designed to provide a better understanding of physiological significance of stress-induced TDP-43 loss of function that can define/signify the earliest pathological changes in a large subset of ALS cases.

123. TDP-43 mutation is associated to intestinal inflammatory phenotypes

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Amyotrophic lateral sclerosis (ALS) is a disease which causes progressive loss of motor neurons and whose patients usually present a fatal outcome within 3-5 years of diagnosis. The absence of specific treatments has led to interest in the investigation of possible genetic and environmental causes that contribute to the progression of the disease. Currently, there are numerous studies demonstrating the involvement of the gut-brain axis and the microbiota in the development and progression of some neurodegenerative diseases, including ALS. Most of these studies are based on mSOD1 in ALS. However, there are few articles that study these alterations in the gut-brain axis in animal models with TDP-43 mutations, which is a key protein in the development of 90% of ALS. Therefore, in this work we investigate the integrity of the intestinal barrier at the expense of the transmembrane cell adhesion protein E-Cadherin and the immune function of Paneth cells by means of the enzyme lysozyme in the ileum of TDP-43 transgenic mice, which are found to be reduced with respect to controls. On the other hand, the histological study of the ileum of TDP-43 mice shows morphological differences with respect to controls. In addition, cytokine levels in ileum, colon, spinal cord and liver have been analysed, finding an increase of IFN-gamma levels in ileum and colon, as well as an increase of IL-6 levels in colon and spinal cord. All these findings suggest the existence of alterations in the intestine-brain axis that trigger the appearance of systemic inflammation, which may contribute to the pathogenesis of ALS.

124. TDP-43 mutations cause disruption in mitochondrial function and axonal transport in ALS iPS-MNs

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A key pathological signature of ALS is the cytoplasmic mislocalization and aggregation of TDP-43 in affected motor neurons, which is found in 97% of cases. Recent reports have shown that mitochondrial dysfunction plays a significant role in motor neuron degeneration in ALS and TDP-43 was found to modulate several mitochondrial transcripts.

Here, we show that motor neurons from induced pluripotent stem cells (iPSCs) carrying the M337V or I383T mutations in TARDBP demonstrate reduced intracellular transport speeds of trafficking organelles. In the presence of glutamate, we detected significantly more stationary TDP-43 mitochondria (95.4%) compared to healthy controls (92.3%). While there are no differences in the percentage of anterograde and retrograde running mitochondria among the genotypes, both TDP-43-M337V (0.051 µm/sec) and TDP-43-I383T (0.053 µm/sec) mitochondria travel retrogradely at significantly lower speeds compared to healthy controls. Analysis of mean velocity of endosomal transport showed a significant reduction in retrograde speed in both patients (0.27 µm/sec and 0.23 µm/sec, respectively) compared to healthy controls (0.38 µm/sec). These deficits also correlate with a decrease in the expression of the motor proteins dynactin-1 and KIF5A. RNA sequencing has shown that transcript KIF5B, one of the anterograde motor proteins, is also reduced in mutant TDP-43-M337V iPS-MNs and we confirmed that protein levels of KIF5B are reduced in TDP-43-M337V MNs after ER stress. Axonal transport is highly dependent on ATP production in the mitochondria. Consequently, we found that mitochondrial bioenergetics, such as ATP production and basal respiration, were significantly reduced in TDP-43 patient iPS-MNs. Additionally, Electron Microscopy imaging revealed an increased fragmentation of mitochondria in patient iPS-MNs compared to healthy controls, which contributed to decreased neuronal viability.

These results indicate that mutant TDP-43 contributes to the reduction in retrograde transport through an imbalance in the expression of anterograde/retrograde proteins and by reducing mitochondrial bioenergetics.

125. TDP-43 splicing function and disease evolution in the FTD-ALS spectrum: Insights from a transgenic mouse model

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Amyotrophic Lateral Sclerosis (ALS) and frontotemporal dementia (FTD) often occur together and both diseases share clinical, pathological, and genetic features. However, the factors that determine which condition predominates during disease evolution remain largely unknown. TDP-43 proteinopathy is a hallmark of the FTD-ALS spectrum, but the role of TDP-43 splicing function in disease evolution is unclear. To investigate this, we used a transgenic mouse model overexpressing a tetO inducible neuron-specific WT hTDP-43 protein. After 8 weeks of doxycycline withdrawal, two different populations were observed in these mice characterized by distinct clinical phenotypes: one with weight gain potentially linked to FTD, and the other with motor dysfunction associated with ALS. We used qPCR assays to analyze TDP-43 splicing function in the brain and lumbar spinal cord (LSC) and quantified p16 mRNA expression, a premature senescence marker. TDP-43 expression and gain of pathological splicing function (assessed by Herc2 skiptic exon inclusion) in LSC are associated with the ALS phenotype, while weight gain is associated with intermediate TDP-43 expression and gain of pathological splicing function. Increased p16 expression in LSC and brain is a specific ALS biomarker, sharing commonalities with the G93A mouse model. Loss of TDP-43 function assessed by Adipor2 cryptic exon splicing was not observed in TDP-43 transgenic mice. These findings suggest that the degree of TDP-43 gain of function influences disease evolution. Furthermore, p16 expression is proposed as a promising ALS biomarker regardless of the disease's genetics. The transgenic mouse model used in this study provides a valuable tool for investigating the FTD-ALS spectrum, identifying novel candidate biomarkers, and uncovering potential therapeutic targets.

126. The cell autonomous effects on sporadic ALS onset and survival

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Amyotrophic lateral sclerosis (ALS) is an insidious disease with poorly understood mechanisms of onset and progression. Especially in sporadic ALS, large variability in disease onset age and survival complicates the insights into causal factors and clinical treatments. Although mechanisms intrinsic to the vulnerable neuronal cells have been widely studied, the roles of processes in surrounding cells are still unclear. We have analyzed gene expression in laser- captured motor neurons (MN) and surrounding anterior horn (AH) tissue and identified respective correlations with age at onset and survival in sporadic ALS patients. These correlating genes were further interpreted with respective ontologies to identify biological processes contributing to onset and survival. Finally, we deconvoluted the correlated genes in the anterior horn using single-cell data to identify potential cell types involved in survival and onset. Our findings show that while expression of genes in motor neurons exhibits multiple correlations with onset age, genes expressed in the anterior horn correlate with both onset age and survival. Interestingly, the genes and ontologies associated with onset and survival were largely non-overlapping, indicating that separate biological processes contribute to disease causality and dynamics. Lastly, we reveal that non-neuronal genes associated with onset age are expressed by endothelium, pericytes and microglia whereas genes associated with survival are representing the mature oligodendrocyte class. This discrepancy further highlights that ALS disease onset and progression mechanisms are likely driven by separate biological processes and cell type categories. Further insight into these relationships could lead to an improved understanding of individual cell type contributions to ALS origins, variability of phenotypic manifestations, and future potential prognostic tools and treatments.

127. The role of ataxin-2 in Drosophila models of C9orf72-related FTD/ALS expressing dipeptide-repeats of a physiologically relevant length

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Introduction: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two devastating, incurable, early-onset neurodegenerative diseases, which share a clinicopathological spectrum of disease; linked by a hexanucleotide (GGGGCC) repeat expansion within the C9orf72 gene. Bidirectional sense and antisense translation of C9orf72 repeat RNA produces five dipeptide repeat proteins (DPRs) which aggregate within neurons, disrupting a range of cellular processes, including stress granule dynamics. It is hypothesised that under conditions of chronic cellular stress, stress granule dysregulation could augment the concentration of aggregation-prone proteins, such as TDP-43, leading to the formation of pathological inclusions, characteristic of FTD/ALS. Furthermore, inhibition of stress granule assembly through ataxin-2 knockdown has been shown to be protective in in vivo TDP-43 models.

Methods: Previously, using Drosophila, we have established the first, and currently only, in vivo models expressing DPRs of a pathologically and physiologically relevant repeat length. Combining primary neuronal cultures and in vivo analysis of these models we investigate the effect of DPRs on stress granule dynamics and the integrated stress response (ISR); and establish whether inhibition of stress granule assembly, through ataxin-2 knockdown, is protective against DPR toxicity.

Results: In select Drosophila DPR models we identify a role for ataxin-2 in DPR-dependent toxicity, observe stress granule accumulation, and identify potential therapeutic targets upstream in the ISR pathway, to mitigate DPR toxicity.

Conclusion: Comprehensive characterisation of the mechanism by which DPR toxicity affects the ISR and stress granule dynamics, is essential to establish therapeutic targets, such as ataxin-2, for C9orf72-mediated FTD/ALS.

128. The Role of Cystatin C in Amyotrophic Lateral Sclerosis

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Bunina bodies are a pathological hallmark specific to amyotrophic lateral sclerosis (ALS). They are observed in the majority of ALS cases at post mortem and are immunoreactive for human Cystatin C (hCC). Under physiological conditions, hCC is present in high cytoplasmic concentrations in lower motor neurons, but free cytoplasmic hCC is lost in many neurons in ALS and hCC-positive Bunina bodies exist within a proportion of remaining lower motor neurons at autopsy. Very little is known about these pathological aggregates in comparison to TDP43 aggregates, the other inclusion body observed in 97% of all ALS cases.

hCC plays a key role in regulating autophagy and has been shown to have anti-amyloidogenic and neuroprotective properties in in vitro and in vivo models. Aggregation is likely to cause the loss of these beneficial functions, and could exacerbate neurodegeneration. Indeed, there is published evidence for the co-occurrence and colocalisation of Bunina bodies and TDP43 aggregates, suggesting a relationship between the two pathological hallmarks. Disruption of autophagy is a known mechanism in ALS, and loss of functional hCC could play a role in this dysfunction, therefore causing or exacerbating TDP43 pathology.

We aim to determine the extent of hCC pathology in sporadic ALS post mortem tissue by immunohistochemistry and to investigate the relationship between hCC pathology and clinical severity, TDP43 pathology and the autophagy marker LC3.

We aim to create in vitro models of hCC loss, and assess the effects on TDP43 localisation and autophagic flux. We then aim to assess whether downstream impacts can be ameliorated by compounds that mimic the action of hCC. Rescuing hCC loss could provide a potential avenue for therapeutic intervention for hCC pathology and ALS.

Results confirm that loss of neuronal cytoplasmic hCC is specific to disease and is not seen in healthy control post mortem spinal cord. By analysing TDP43 and hCC in serially stained sections, TDP43 pathology appears related to the loss of cytoplasmic hCC: loss of nuclear TDP43 only occurs in hCC immuno-negative neurons, implying a relationship between the pathological hallmarks of ALS.

129. The Role of Microglia-released microRNAs in ALS Pathology

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Microglia, the resident immune cells in the CNS, play a key role in driving neuroinflammatory responses and disease progression in ALS. Microglia adopt a unique and dynamic phenotype in ALS, releasing a plethora of cytokines and signals, including microRNAs (miRNA). There is increasing evidence that released miRNA play an important role in cell-to-cell communication, by modulating gene expression in recipient cells. There is, however, a significant gap in our knowledge about the identity of microglia-derived miRNA and their impact on motor neurons, in the context of ALS pathology. We hypothesise that microglia in the CNS of people with ALS release a distinct miRNA signature that causes detrimental changes to motor neuron gene expression and contributes to motor neuron degeneration.

To address this hypothesis, we have cultured primary neonatal microglia from hippocampal and cortical brain regions of hSOD1-G93A transgenic mice and two control models: hSOD1-WT transgenic mice and Non-Transgenic (NTg) mice. Microglia were incubated for 24 hours with lipopolysaccharide (LPS), interleukin-4 (IL-4), or vehicle control. We sequenced the miRNA released into cell culture media over the 24-hour period and used negative binomial models to explore differential miRNA expression between genotypes. 23 miRNA were significantly differentially regulated between hSOD1-G93A mutants and either or both controls when treated with LPS, whereas 36 miRNA were significantly differentially regulated when treated with IL-4. We then explored dysregulation attributable to an interaction between genotype and treatment, while controlling for variation between mice, and found 73 dysregulated miRNA with LPS treatment but only 10 with IL-4 treatment. Quantitative PCR validation indicated that some miRNA released from microglia are dysregulated with treatment, including the pro-inflammatory miR-155-5p, and others also vary between genotypes, including miR-320-3p.

We are now exploring the effects of these differentially expressed microglia-released miRNA on mouse embryonic stem cell-derived motor neurons. We will present survival, morphological and miRNA target gene expression data from motor neurons transfected with microglia-derived miRNA. This work will further our understanding of the pathological cell-to-cell communication pathways that may contribute to motor neuron degeneration in ALS.

130. The role of Serum Response Factor (SRF) in the regulation of autophagy

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Neuronal activity plays a crucial role in motoneuron (MN) vulnerability in amyotrophic lateral sclerosis (ALS). Enhanced MN excitability has been shown to promote neuroprotection while reduced excitability accelerates disease progression. However, the molecular basis of neuronal activity's impact in ALS has not been identified yet. In this study, the impact of the activity-dependent transcription factor Serum Response Factor (SRF) was investigated in vivo and in vitro. Conditional MN-selective SRF ablation in the context of the SOD1(G93A) ALS mouse model caused an earlier disease onset as revealed by enhanced body weight loss, earlier appearance of advanced clinical stages and faster decline in grip-strength. However, overall survival was not affected, indicating a major role of SRF only for some MN subpopulations. At P50, loss of SRF in MNs caused a more pronounced neuromuscular junction denervation and augmented neuroinflammatory response. Increased vulnerability of MNs corresponded with fewer Beclin positive inclusions in histology and reduced induction of autophagy genes assessed in laser-microdissected MNs. We complemented these studies assessing autophagy genes induction in HEK293 cells expressing the C9-Orf72-associated polyGA polypeptide: the overexpression of SRF resulted in a decreased burden of polyGA inclusions. Live-imaging microscopy with the autophagy sensor P62-GFP-RFP revealed a faster autophagy flux upon overexpression of SRF compared to an inactive SRF mutant protein. Thus, in vitro data further confirmed a role of SRF in the transcriptional regulation of cellular proteostasis. Chemoenetic manipulations of neuronal activity uncovered SRF as an important transcription factor mediating activity-dependent effects on ALS associated autophagy and disease burden. In conclusion, our data identify with SRF a new gene regulator connecting neuronal activity with the cellular autophagy program initiated in degenerating MNs

131. Therapeutic potential of parthenolide in the SOD1-G93A mouse model of Amyotrophic Lateral Sclerosis?

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Therapeutic effects of natural substances have become more and more popular over the last two decades and have thus increasingly become a topic of research - especially in so far incurable diseases such as ALS. The sesquiterpene lactone parthenolide (PTL), a which occurs naturally in the plant feverfew (Tanacetum parthenium has been explored as a potential treatment for migraine, showed promising therapeutic potential against glioma and broad-spectrum anti-cancer activity as well as stimulation of peripheral nerve regeneration.

Our previous work with primary microglial cells derived from mutant SOD1-G93A mice shows promising results on microglial phenotype markers like inducible nitric oxide synthases or tumor necrosis factor alpha. We also found positive impact of PTL treatment in co-cultures of primary motor neurons and microglia cells of SOD1-G93A mice. In light of these results and what is known from the literature, we then investigated PTL as a potential treatment for ALS in the SOD1-G93A mouse model.

Treatment was initiated before symptom onset in order to assess the general neuroprotective potential of PTL. Two groups of transgenic animals received PTL in different concentrations while the control group received the carrier substance via drinking water.

A first group of animals was tested by neurosonography and electrophysiological studies. Here we detected positive effects of PTL on the cross-sectional area and nerve conduction velocity of the sciatic nerve. Animals are currently further assessed by motor function tests, phenotypic analyses and survival analyses. Preliminary results show a delay in disease onset and better general condition in PTL-treated animals, but so far no impact on survival.

132. THERAPEUTIC POTENTIAL OF THE NRF2 ACTIVATOR DIMETHYL FUMARATE IN TDP-43-RELATED FRONTOTEMPORAL DEMENTIA MOUSE MODEL

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Frontotemporal dementia (FTD) is a neurodegenerative disease characterized by progressive degeneration of frontal and temporal lobes of the brain, leading to behavioral, cognitive and language impairments. Since there is not an effective treatment, it is necessary to look for new therapeutic strategies for this disorder. Recently, the transcription factor NRF2 was found to be a crucial element in restricting neurodegeneration. Actually, its pharmacological activation may mitigate multiple pathogenic processes involved in FTD, such as oxidative stress, inflammation and maintenance of protein homeostasis. In previous studies, it has been demonstrated the neuroprotective effects of dimethyl fumarate (DMF), a NRF2 activator, in a TAU-dependent FTD murine model, by decreasing neurodegeneration, neuroinflammation, oxidative stress and TAU hyperphosphorylation. Therefore, these results support the reposition of DMF for the treatment of FTD. Additionally, TAU overexpression causes TDP-43 delocalization from the nucleus to the cytoplasm, whereas TDP-43 relocated back to the nucleus with DMF treatment. However, the efficacy of this treatment for TDP-43-FTD has not been investigated yet. Therefore, the main aim of this research was to study the therapeutic effects of the pharmacological activation of NRF2 with DMF in a TDP-43-related frontotemporal dementia mouse model which overexpresses TDP-43 protein under α-CaMKII promoter, leading to early cognitive impairment and other behavioural abnormalities. Mice were treated every other day with the DMF (i.g. 100 mg/kg) from presymptomatic stages (PND45) to symptomatic phases (PND90). Two weeks after starting the chronic treatment (PND60), FTD mice treated with DMF displayed an improvement in cognitive function, reflected in an increased in the discrimination index and in the exploration time of the new object in the Novel Object Recognition (NOR) test. Notably, this effect was even more prominent at the end of the treatment (PND90). In conclusion, our data confirm that the modulation of NRF2 with DMF preserves cognitive impairment in a TDP-43-related FTD mouse model.

133. Transcriptomics Analyses of ALS Postmortem Motor Cortex highlight alteration and potential biomarkers in the Neuropeptide Signalling pathway

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal heterogeneous neurodegenerative disease that typically leads to death from respiratory failure within two to five years. Despite the identification of several genetic risk factors linked to the disease, the biological processes involved in the ALS pathogenesis remain poorly understood.

Methods: Using two independent bulk total RNAseq datasets from postmortem motor cortex samples of ALS patients and controls, King's College London (KCL) BrainBank (171 subjects) and TargetALS (132 subjects), we performed a large differential expression study of this key ALS brain region. We used a wide range of biological databases to perform gene set enrichment analyses of the differentially expressed genes in the two datasets and compared the results. We also reviewed the role of the most significant differentially expressed genes in both datasets in the context of the pathways and processes highlighted by the enrichment analyses and selected a set of candidate target genes. Finally, we investigated whether expression levels of the candidate genes correlate with patient clinical measures.

Results: The Neuropeptide signalling pathway was highly significant in both the KCL BrainBank and TargetALS datasets. We investigated whether neuropeptides and their receptors were linked to the clinical phenotype and identified a set of them that correlated with age of onset and disease duration in both datasets. GO process enrichment revealed significant synapse-related processes in the KCL BrainBank dataset, while the TargetALS dataset carried an immune system related signature. We provide access to gene-level results to the broader research community through a publicly available web application (https://alsgeexplorer.rosalind.kcl.ac.uk/), allowing researchers to query the expression of specific genes in the motor cortex of ALS cases and non-affected controls in the two datasets.

Conclusion: This study identified pathways that are consistently altered in two independent motor cortex datasets of ALS patients, potential molecular targets for therapeutic intervention, and suggested a set of neuropeptides and receptors for investigation as potential biomarkers.

134. U1 snRNA as a novel RNA-based therapeutic approach to modulate C9ORF72 pathology in patient-derived iPSC-motoneurons

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A novel class of RNA-based therapeutic molecules is represented by modified spliceosomal U1 small nuclear RNAs (snRNAs) which, by acting on pre-mRNA splicing, have already proved to be effective in mice models of spinal muscular atrophy and Familial dysautonomia.

Aim of this work is to test whether modified U1 snRNAs, designed to bind the C9ORF72 hexanucleotide repeat expansion (HRE), correct C9ORF72-associated pathology as a valuable and alternative RNA-based therapeutic strategy to antisense oligonucleotides in preclinical ALS patient-derived cell models such as iPSC-motoneurons (iPSC-MN).

Two different modified U1 snRNAs (U1C and U1G) were preliminarily transfected in HEK293T cells overexpressing 66 hexanucleotide repeats. FISH analysis showed that both U1 constructs significantly decreased the mean number of pathological RNA foci per cell. To assess whether U1C and U1G had an effect also on RAN translation of the HRE-containing transcripts, we used a nonATG-plasmid with the 66-hexanucleotide repeat upstream a GFP tag. A significant reduction also in the formation of polyGP-GFP proteins was observed upon transfection with both modified U1s. We then tested the efficacy of U1 snRNAs in reducing RNA foci formation in C9ORF72 patient-derived iPSC-MN carrying 1200 repeats by lentiviral-mediated delivery of U1s. FISH analysis showed a significant decrease both in the percentage of cells containing pathological RNA foci and in the number of RNA foci per cell.

We are currently confirming our data in other C9ORF72 iPSC lines carrying different HRE size (150 and 670 units) to assess if U1s efficacy in reducing RNA foci is length-dependent.

Our results suggest that modified U1 snRNAs targeting the pathological HRE represent an innovative therapeutic strategy to treat ALS and FTD patients carrying C9ORF72 mutation.

135. Ultra-high spatial resolution ex-vivo structural brain MRI reveals early caudoputamen atrophy in FusΔNLS/+ mice

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FUS protein is one of the key factors of ALS/FTD spectrum disorder. The mislocalization of the full-length FUS protein from nucleus to cytoplasm and its aggregates are characteristic of a subset of aggressive sporadic FTD forms. However, the driving role of FUS in FTD, anatomical substrate and mechanisms remains unclear, in part due to resolution drawbacks in current volumetric imaging.

Here we applied a newly developed strategy for ultra-high, spatial resolution ex-vivo magnetic resonance imaging (MRI) in FusΔNLS/+ mice that replicate some key features of ALS/FTD, to investigate brain structural abnormalities related to the disease.

MRI was performed on fixed brains stored in Fluorinert using an ultrahigh field 11.7T small animal system. Data were acquired using a cryogenically cooled coil together with optimized imaging parameters to provide highest contrast and to delineate maximum substructures at scans separated for only 40um. In house developed image analysis pipeline matched the MRI dataset to anatomical plates from the Allen Brain Atlas and provided automated measure of brain structures' volume with a discrimination possible at the cortical layer precision.

We demonstrate that as early as the first FTD-like behavioural symptoms occur in FusΔNLS/+ mice at 6 months of age, MRI volumetric analysis is sufficiently sensitive to detect structural correlates such as subcortical atrophy, whereas caudoputamen is affected first. With disease progression, at 12 months of age, when FusΔNLS/+ mice manifest FTD- and ALS-like symptoms, MRI detects spreading of atrophy involving multiple subcortical (deep cerebral nuclei due to caudoputamen and pallidum, but also hypothalamus) and cortical structures (isocortex due to motor areas, then pirform and retrosplenial areas), despite no change in overall brain volume. This is justified by a significant increase in volume of lateral ventricles. The hierarchical cluster and principal component analysis reveal that characteristic MRI phenotype is sufficient to separate animals into groups according to their genotype and suggest relevance of structural abnormalities for the disease. Cross-correlation analysis show related atrophy of motor areas and of deep cerebral nuclei.

Overall, novel ex-vivo MRI method provides histology-grade resolution and enables, in FusΔNLS/+ mouse model, to detect the pattern of atrophy of brain structures as a proxy of their earliest vulnerability beyond what was previously demonstrated.

136. Ultrastructural Imaging of Distinct SOD1 Strains in ALS Transgenic Mouse Models

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Abnormally assembled proteins play a central role in neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's and Amyotrophic lateral sclerosis (ALS). In these diseases, evolving evidence show the capability of misfolded proteins to assemble into different strains and propagate in a prion-like manner. Moreover, several studies suggest the existence of conformational strains of misfolded aggregates may account for the large heterogeneity of symptoms within these diseases. The presence of aggregates formed by misfolded superoxide dismutase-1 (SOD1) proteins are hallmarks of ALS caused by mutations in the SOD1 gene. In our laboratory, two structurally distinct strains of human (h) SOD1 aggregates (denoted A and B) have previously been identified in transgenic (Tg) hSOD1 mice. Intra-spinal inoculations of these strains into adult mice have shown their capability to induce strain-specific aggregation through templating and cause premature fatal motor neuron disease, supporting that ALS caused by SOD1 mutations is a prion-like disease. Additionally, it was shown that strain A and B affect the progression of the disease differently, suggesting a difference in pathogenesis provoked by the distinct strains. These specific pathological mechanisms of the structurally different hSOD1 strains have previously not been determined. In this study, we optimized a protocol to detect strain A and B aggregates at a subcellular level in order to investigate their pathological effects. Antibodies targeting strain specific sequences were used to label hSOD1 aggregates in spinal cords of hSOD1G85R and hSOD1D90A Tg mice. The aggregates were visualized using correlative light-electron microscopy allowing investigation of the aggregates within their cellular environment. Our results show a successfully developed protocol for ultrastructural visualization of strain A and the next step will be to image strain B. These findings form the basis to further investigate whether conformational changes within SOD1 fibrils can alter the pathological function of the protein and be linked to specific variants of ALS.

137. Unconventional secretion of misfolded SOD1 and toxicity spreading: a novel therapeutic strategy for ALS

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OBJECTIVES: Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of motoneurons leading to paralysis and death. Among the familial forms of the disease (10%), the first gene identified codes for an ubiquitous protein, superoxide dismutase type 1 (SOD1). Transgenic mice expressing mutated human forms of SOD1 faithfully summarize the main features of the disease. Insufficient degradation of these aberrant proteins induces a gain in intracellular toxic function in motoneurons. However, numerous studies have shown that the loss of motor functions is due to a combination of deleterious non-cell autonomous mechanisms encountered in many cell types, involving the spread of toxic molecules such as mutant SOD1 in a prion-like fashion. Our objective is to understand the role of unconventional secretion pathway for misfolded proteins in SOD1-related ALS.

METHODS & RESULTS: Through the description of unconventional secretion mediated by the ubiquitin specific protease USP19, we study the secretion of SOD1G93A mutant using cell culture system and expression of functional mutants. The analyzis of UPS19 expression pattern is done at different disease stages by immunofluorescence and biochemistry in SOD1G93A mice. Besides, innovative technologies are validated to silence efficiently USP19 in vitro. Our data show that the USP19 promotes the secretion of SOD1G93A initiating its loading at the endoplasmic reticulum membrane. We found that USP19 is predominantly expressed in oligodendrocytes and show differential expression levels in ALS mice.

CONCLUSION: These results provide new knowledge on the proteinopathy aspect of the ALS and pave the way for the preclinical evaluation of a targeted intervention in ALS mice.

138. UNCONVENTIONAL SECRETION OF TDP-43 FREE AGGREGATES BY THE UBIQUITIN-SPECIFIC PROTEASE 19 (USP19)

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Amyotrophic Lateral Sclerosis (ALS) is a proteinopathy characterized by the presence of pathological inclusions that are mainly composed of ubiquitinated, hyperphosphorylated and cleaved TDP-43. Different studies revealed that pathological TDP-43 can be released in the extracellular space in association with extracellular vesicles (EVs) or as free aggregates. However, little is known about the cellular mechanisms by which free TDP-43 aggregates can be secreted. Recently, a new unconventional secretion pathway mediated by the endoplasmic reticulum (ER)-resident-deubiquitinase Ubiquitin-Specific Protease 19 (USP19) was identified in the release of α-Synuclein and Tau pathological proteins.

Here we investigated the role of USP19 in the context of wild type (WT) and mutant K263E-TDP-43. We found that USP19-WT expression faintly induces the release of overexpressed WT-TDP-43 in the extracellular space, whereas in the same context, a strong secretion of the K263E-TDP-43 was observed. Conversely, the expression of its non-ER-resident USP19ΔTM or its deubiquitinase inactive (USP19-C506S) mutants failed to induce this release. Analyses of conditioned media from USP19-WT/K263E-TDP-43 co-expressing cells through sucrose density gradient fractionations revealed that K263E-TDP-43 was moderately detected in positive EVs fractions but was highly enriched in dense fractions (1.20-1.26 g/cm3) possibly corresponding to the presence of more compacted structures. Immunogold electron microscopy (IEM) analyses confirmed the presence of heterogeneous globular and fibrillar compacted structures, labelled by a gold anti-TDP-43 antibody thus indicating that USP19-WT expression favors the release of TDP-43 free aggregates.

Interestingly, a significant increase of lipidated-LC3 was observed in the USP19-WT/K263E-TDP-43 co-expressing cells and correlated with the detection, by IEM, of TDP-43 in autophagic-like compartments. Inhibition of fusion between autophagosomes and lysosomes compartments by Bafilomycin A1 or chloroquine inhibitors failed to impair the release of K263E-TDP-43 aggregates. Conversely, inhibition of autophagosome biogenesis using the spautin-1 inhibitor strongly impaired their released.

Taking together, our data revealed that USP19-WT expression strongly enhances the release of K263E-TDP-43 free aggregates in the extracellular space through a cellular trafficking pathway involving early autophagic compartments.

139. Uncovering the protein interactions of MATR3 and its ALS-associated mutant MATR3 S85C

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Introduction

Matrin 3 (MATR3) is a DNA/RNA-binding nuclear matrix protein involved in several cellular processes. These include early stages of DNA damage response, transcription, mRNA stability, alternative splicing, and mRNA export. Mutation S85C in the MATR3 gene is associated with the development of a slowly progressive form of amyotrophic lateral sclerosis (ALS). However, the role of MATR3 in the pathogenesis of this disease is unknown. Moreover, little is known about the protein-protein interactions of MATR3 and its mutants.

Objectives

In the present study, we aimed to identify proteins that interact with human MATR3 and its ALS-mutant MATR3 S85C in vivo.

Methods

To this end, we generated mammalian cell lines stably expressing the fusion protein of MATR3 or MATR3 S85C with the biotin ligase BioID2 or BioID2 without a fusion partner. After induction of BioID2 activity, the biotinylated proteins were isolated from the cell lysates by pull-down assay. The isolated proteins were then detected by Western blotting, silver staining, and liquid chromatography – mass spectrometry (LC-MS). Analysis of LC-MS revealed a list of unique MATR3 and MATR3 S85C interactors. The interactors were further investigated by bioinformatics analysis.

Results

The BioID method was used to uncover the unique protein interactions of MATR3 and its

mutant MATR3 S85C. We found that the fusion proteins of both MATR3 variants with the biotin ligase BioID2 have the same cellular localization as endogenous MATR3 and that BioID2 is enzymatically active. Through a successful pull-down assay, we isolated biotinylated interaction partners of MATR3 and MATR3 S85C. Using silver staining and immunodetection of the biotinylated proteins after Western blotting, we detected proteins specific for samples with MATR3 and MATR3 S85C but not for samples with BioID2 alone. We then identified these interaction partners by LC-MS. Among the identified proteins were already known interactors of MATR3 and its mutant S85C as well as new, yet unknown ones that could contribute significantly to the development of ALS.

Conclusion

Our study suggests that MATR3 and its ALS-mutant MATR3 S85C have both shared and distinct protein interactors. Understanding the changes in the interactome of both MATR3 variants could provide valuable insights into the role of MATR3 in the pathology of ALS and pave the way for new therapies and biomarker discovery.

140. Uncovering the proteome of TDP-43 condensates in human neurons

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Cytoplasmic TDP-43 aggregates are found in inclusions in the vast majority of ALS neuropathology specimen. However, other than TDP-43 itself, the protein composition of these macromolecular assemblies is unknown and a systematic investigation of human, neuron-specific TDP-43 interactors was never performed. Mapping the composition of TDP-43 condensates inside human motor neurons will promote understanding of the mechanisms of disease and may identify potential therapeutic targets. In this research, we implement APEX proximity proteomics for mapping of proteins interacting with TDP-43 in the cytoplasm of human motor neurons. Using unbiased mass spectrometry, we reveal both known and novel protein interactors of TDP-43. Surprisingly, many proteins map to the centrosome and P-body compartments. Moreover, upon sodium arsenite stress, a subset of interactions is lost and a unique set of proteins is recruited inside TDP-43 positive condensates. Many kinesins specifically are enriched inside TDP-43 condensates. Overall, this study uncovers novel TDP-43 protein interactions in the cytoplasm as well as changes in these interactions upon condensation. The latter may aid understanding of the pathophysiological consequences of cytoplasmic TDP-43 relocalization in ALS.

141. Understanding muscle impairment in Amyotrophic Lateral Sclerosis in the absence of motoneuron input

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ALS, the most common motor neuron disease, is today defined as a multisystem disorder that includes changes in structural, physiological and metabolic parameters in different cell types. In this sense, defects have been reported at the neuromuscular junction (NMJ) level, as well as intrinsic muscle abnormalities. Trying to elucidate whether the skeletal muscle is involved in the pathogenesis of the disease, ALS patient-derived myoblasts and skeletal muscle samples are being characterized in our group. Our results indicate that the expression of the myogenic regulatory factors MYOD and MYOG are reduced, while atrophy markers such as Atrogin-1 and MuRF1 are increased. Importantly, the differentiation capacity of patient-derived myoblasts is diminished, suggesting an impairment in muscle regenerative function. Strikingly, differentiation indexes correlate with the proportion of nuclear TDP-43 or FUS expressed in primary myoblasts. Besides, we are currently characterizing patient-derived muscle tissue regarding myogenesis and ALS-related proteins such as TDP-43 and FUS, as well as the state of the NMJ. Attempting to model the pathological events occurring in ALS muscle, we have performed silencing of TARDBP (TDP-43) and FUS in human immortalized myoblasts confirming the myogenic defects observed in patient samples. Together, we believe that our results and others achievable through the use of such in vitro model would aid in the better understanding of the mechanisms involve in the disease which may lead to the discovery of novel targets with therapeutic potential for ALS.

142. Understanding the neuropathological consequences of NEK1 mutations in ALS

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NEK1, or (never in mitosis gene-A)-related kinase 1, is a serine/threonine kinase with loss-of-function and missense variants that have recently been associated with an increased risk of ALS. However, the cellular consequences of NEK1 mutation, as well as the clinical and pathological phenotypes related to specific NEK1 variants, remain undescribed, as no study thus far has pathologically examined post-mortem tissue from these rare cases. Here we set out to undertake the first characterisation of neuropathological phenotypes associated with NEK1 mutations in ALS, in a case series. Our study characterises post-mortem tissue from three Scottish patients with a NEK1 mutation who went on to develop ALS. Using immunohistochemistry, we evaluated the expression and distribution of NEK1 as well as phosphorylated TDP-43 (pTDP-43) aggregates, a pathological hallmark of ALS, in the motor cortex and amygdala. We additionally examined the abundance and distribution of NEK1 mRNA molecules using BaseScope™ in situ hybridisation. For one case with a p.Arg261His missense mutation, we observed increased NEK1 mRNA expression and abundant NEK1-positive cytoplasmic aggregates, with the same morphology and distribution as the co-occurring pTDP-43 aggregates. By contrast, the other two NEK1-ALS cases exhibited reduced NEK1 mRNA expression and an absence of NEK1-positive cytoplasmic aggregates, indicating loss of NEK1 function. Our findings suggest a spectrum of clinical and neuropathological consequences for NEK1 variants in ALS.

143. Unrevealling the molecular mechanisms involved in the protection exerted by genetic leptin haploinsufficiency in the mouse model of ALS SOD1G93A

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Amyotrophic lateral sclerosis (ALS) is a multifactorial and multistep fatal degenerative disorder. Both patients and animal models show significant dysregulation in metabolism such as body weight loss, altered lipid levels and hypermetabolism. Leptin, one of the main metabolic related hormones, is altered in this pathology. A previous study observed that a genetic haploinsufficiency of leptin (Lepob/+) in an ALS mouse model (SOD1G93A) improves survival and motor performance, although the molecular mechanism behind these beneficial effects was not studied. Here, we aim to identify the molecular pathways that are responsible for these beneficial effects by transcriptomic RNA-sequencing of the lumbar spinal cord and the subcutaneous inguinal white adipose tissue (iWAT) in SOD1G93A-Lepob/+ mice. The transcriptomic results suggested that the leptin haploinsufficiency in the iWAT of SOD1G93A-Lepob/+ mice reduced the immune response, since most lymphocyte activation, differentiation and proliferation genes were downregulated. However, in the spinal cord, the numerous neuroinflammatory alterations that occur in the SOD1G93A mice were not reverted by genetic leptin haploinsufficiency. In conclusion, this data suggest that the protective effects of leptin depletion in SOD1 neuromuscular pathology may be related to the modulation that leptin exerts on the immune system outside of the central nervous system tissues.

144. Using optogenetics to model activity-dependent neurodegeneration in amyotrophic lateral sclerosis

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Background: Increasing evidence suggests that abnormal activity and excitotoxicity underlie the selective vulnerability of motor neurons (MNs) in amyotrophic lateral sclerosis (ALS). The C9orf72 hexanucleotide repeat expansion (HRE) is the leading genetic cause, linked to hyper- and hypo- excitability and glutamate toxicity in induced pluripotent stem cell (IPSC)-MNs. However, mechanisms of this are unclear, and immaturity of IPSC-MNs prevents us from resolving whether these are features of early or late disease. To address this requires control of MN activity. We utilised optogenetic IPSC-MNs to answer these questions.

Aims: To study optogenetically stimulated IPSC-MNs alone and in co-culture with muscle to characterise effects on maturity and mechanisms of activity-dependent degeneration in C9orf72-ALS.

Methods: We differentiated IPSC-MNs from C9orf72-ALS patients, isogenic and healthy controls. We characterised activity using multi-electrode array (MEA). IPSC-MNs expressing channelrhodopsin (ChR2) underwent chronic low- or high-level light stimulation to find effects on (i) IPSC-MN maturity and (ii) mechanisms of C9orf72-ALS. ChR2 IPSC-MNs and C2C12 muscle co-cultures were developed to assess C9orf72 HRE influence on the neuromuscular junction (NMJ) and muscle.

Results: Low level stimulation of non-mutant IPSC-MNs led to increased choline acetyltransferase expression. MEA analysis revealed no differences in firing rate, but reduced bursting in C9orf72 IPSC-MNs relative to controls, and an absence of network activity. High level chronic stimulation of ChR2-C9orf72 HRE IPSC-MNs induced MN death (relative to controls), partially rescued by CRISPR-Cas9 correction of the HRE. Stimulated C9orf72 IPSC-MNs exhibited C9orf72 haploinsufficiency relative to controls. In healthy IPSC-MNs, stimulation induced a significant increase in lysosomal marker LAMP1, but not in C9orf72 IPSC-MNs. We studied IPSC-MN muscle co-cultures for effects of C9orf72-HRE on NMJ and light-controlled contraction.

Conclusions: We provide preliminary evidence that optogenetic stimulation enhances IPSC-MN maturity. We also show intrinsic abnormal activity of C9orf72-IPSC-MNs and vulnerability due to activity-dependent C9orf72 loss of function and lysosomal dysfunction. These stimulation-dependent findings provide support for this novel application of optogenetics as a platform for further study of pathways to MN and NMJ degeneration in ALS.

145. Utreloxastat, a Novel 15-Lipoxygenase Inhibitor, Reduces Ferroptosis-Induced Injury for ALS and Related Neurodegenerative Diseases

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Background: Ferroptosis is a form of iron- and lipid-dependent regulated cell death associated with lipoxygenase-mediated lipid peroxide production and glutathione depletion. Activation of ferroptosis has been implicated in a growing number of disorders, including amyotrophic lateral sclerosis (ALS). Because ferroptosis is regulated by balancing the activities of glutathione peroxidase 4 (GPX4) and 15-lipoxygenase (15-LO), targeting 15-LO offers a promising approach for treating ALS.

Methods: To assess the effectiveness of utreloxastat 15-LO inhibition on cell survival in vitro, SOD1 mouse or human spinal astrocytes were subjected to a pro-ferroptotic challenge (RSL3-induced GPX4 inhibition and iron oxidation by FINO2) in the presence of utreloxastat. Three approved ALS medications (riluzole, relyvrio, and edaravone) were also evaluated. To determine the effects of utreloxastat on survival, 4-week-old SOD1-G93A mice were dosed daily for 16 weeks, and clinical scores were recorded each day (n = 16 animals/arm). Whole blood concentration of 15-LO product, 12-hydroxyeicosatetraenoic acid (12-HETE), was measured in healthy C57B/6 mice following a single-dose of utreloxastat (10-300 mg/kg) to assess target engagement and dose selection.

Results: Utreloxastat treatment prevented FINO2- and RSL3-induced ferroptotic cell death of spinal astrocytes, with cellular potency (EC50) values ≤250 nM and <98.9 nM, respectively, and showed no apparent effect on cell viability at concentrations ≥5000 nM after prolonged (96 hours) treatment. Neither riluzole nor relyvrio conferred protection (≤5000 nM and ≤30000 nM, respectively), and utreloxastat was 70x more potent than edaravone (7000 nM) in preventing RSL3-induced ferroptotic death . In the SOD1 study, utreloxastat-treated animals had improved grip strength and survival compared to vehicle-treated animals. Utreloxastat exhibited a time- and dose-dependent decrease in 12-HETE in mice with a median effective dose (ED50), which elicited a 50% decrease in 12-HETE levels estimated at 41 mg/kg.

Discussion: In totality, these data provide compelling evidence for targeting 15-LO and ferroptosis as a novel method for treating ALS. After completion of the requisite safety studies and regulatory filings, utreloxastat completed a Phase 1 testing in healthy adults and is currently being tested in a Phase 2 placebo-controlled randomized trial to assess efficacy and safety in ALS patients (CARDINALS; NCT05349721).

146. Validation and characterisation of the therapeutic effect of HDAC6 inhibition using a zebrafish model for ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease characterised by the degeneration of upper and lower motor neurons (MNs) followed by complete paralysis and death of the patient usually within 2 to 5 years after diagnosis. The devastating lack of effective treatments underlines the urgent need for further research towards the development of new therapeutic interventions. Our lab discovered that histone deacetylase 6 (HDAC6) could be a promising therapeutic target as its inhibition ameliorates ALS-related phenotypes in various in vitro disease models. More specifically, HDAC6 inhibition rescues axonal transport defects as well as impaired neurite outgrowth in induced pluripotent stem cell-derived MNs from ALS patients harbouring mutations in ALS-associated genes (FUS, TDP-43...). HDAC6 plays a unique role in many biological processes linked to ALS. However, little is known about the mechanisms underlying the observed beneficial effects of HDAC6 modulation. Therefore, the main aim of this study is to translate our in vitro findings into an in vivo zebrafish model for ALS. To do this, we investigated in vivo whether pharmacological inhibition and/or genetic silencing of HDAC6 rescues the observed phenotypes in our human FUS-ALS embryonic zebrafish model. After injecting mRNA encoding for different mutated variants of the human FUS protein (P525L, R521H, R521G) into one-cell stage zebrafish embryos, we characterised ALS-related phenotypes at 48 hours post fertilisation. We discovered axonal outgrowth to be impaired in the ventral root axonal projections of spinal cord MNs in live embryos. Additionally, an ALS-like motor phenotype based on the swimming behaviour of the embryos was observed. When treating the FUS-injected zebrafish embryos with a selective HDAC6 inhibitor, both phenotypes improved significantly. Furthermore, genetic silencing of hdac6 (zebrafish orthologue for HDAC6) with antisense morpholino oligonucleotides also improved the ALS-related phenotypes in the FUS-ALS zebrafish model. Following the establishment of this model as a screening tool, this study further aims to elucidate the exact role of HDAC6 in ALS by identifying and investigating its relevant key interactors.

147. Validation of Exportin-1 (XPO-1) and Mitogen-Activated Protein Kinase Kinase 2 (MAP2K2) as Molecular Drug Targets in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder and effective therapeutic options are lacking. Profiling ALS pathomechanisms could assist with the development of new treatment avenues. Therefore, this study aimed to validate molecular candidates with potential implication in ALS pathology which were derived from multiomic profiling studies conducted in the context of the MAXOMOD consortium (Multiomic analysis of axono-synaptic degeneration in motoneuron disease) (Abstract from Caldi Gomes, et al. ENCALS 2023). Based on differential expression results and functional enrichment analyses, Exportin 1 (XPO-1) and mitogen-activated protein kinase kinase 2 (MAP2K2) were selected to be validated in vitro. XPO-1 is a major regulator of nuclear RNA export and MAP2K2 has an important role in various cell functions, including neuronal differentiation and survival. Multiomic analysis of human postmortem prefrontal cortex (PFC) and PFC from transgenic ALS mouse models revealed multiple deregulated molecular targets and pathways underlying the degeneration of ALS-affected brains. To validate selected molecular targets, we established primary cortical neuron cultures from P0 C57/Bl6 mice. To assess the role of the targets on neuronal survival we used in vitro toxin models mimicking known disease pathways in ALS, such as glutamate excitotoxicity and arsenite-induced stress granule formation. Basal expression of the targets (XPO-1 and MAPK2K2) and toxicity/functionality of the inhibitors (selinexor and trametinib) were investigated by Western blot. Neuroprotective effects of target inhibition were investigated in toxin models by immunocytochemistry (cleaved caspase-3) and analysis of neurite outgrowth using imageJ. We modulated the expression of XPO-1 and MAP2K2 with FDA-approved pharmacological small molecule inhibitors (selinexor and trametinib). Our results demonstrated that 72h treatment with 20nM and 200nM trametinib, completely restored overexpressed phospho-Erk1/2 protein which is a direct target of MAP2K2 ($n=3$; $P < 0.05$) and significantly increased average neurite length in glutamate-intoxicated cells ($n=4$; $P < 0.0001$). 10nM selinexor on the other hand, significantly reduced cell death in stress granules induced cultures ($n=4$; $P < 0.05$) but didn't affect cell survival in glutamate excitotoxicity model. Our findings suggest that XPO-1 as well as MAP2K2 could be auspicious drug targets to be validated for the treatment of ALS.

148. Whole-genome bisulfite sequencing of motor neurons reveals cell specific enhancers & enables the identification of motor neuron derived cell free DNA

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Background

The cardinal feature of amyotrophic lateral sclerosis (ALS) is the loss of upper and lower motor neurons, which causes progressive weakness and death. Vital to understanding the mechanism of ALS and finding successful treatments is understanding the selective vulnerability of motor neurons. A key regulator of cell specificity is cell specific epigenetic modifications which enable expression of cell specific genes.

In order to understand the unique epigenetic regulation in motor neurons we performed whole genome bisulfite sequencing (WGBS) in induced pluripotent stem cell (iPSC) derived motor neurons to capture genome wide methylation state. We then analysed these methylation profiles alongside a DNA methylation atlas of cell types from Loyfer et al. (2023) to identify motor neuron specific hypo and hypermethylated regions. We then analysed these regions to identify motor neuron specific regulatory regions such as enhancers.

A key use of a reference methylome is that it can be used with a deconvolution algorithm to infer the cellular composition of a mixture from its methylation state. Cell free DNA (cfDNA) in blood plasma is composed of DNA from recently lysed cells, and quantifying the motor neuron derived DNA within that has previously been identified as a promising biomarker in ALS (Robichaud et al. 2021). We used our data to test the feasibility of motor neuron-derived cfDNA as a biomarker

Objectives

To identify motor neuron specific hyper and hypomethylated regions, then to use these regions to identify motor neuron specific enhancers and regulatory regions.

Test the feasibility of motor neuron derived cfDNA as a biomarker of ALS.

Methods

We integrated whole genome bisulfite sequencing of iPSC-derived motor neurons derived from three healthy donors with the methylation atlas from Loyfer et al. (2023) to identify motor neuron specific regions.

We optimised a deconvolution algorithm using synthetic mixes of cfDNA from healthy donors and motor neuron derived DNA and describe its accuracy.

Finally, we will use our optimised deconvolution algorithm on WGBS sequenced cfDNA from 12 ALS patients and 12 controls, previously published in (Caggiano et al. 2021).

Results

We identify 5281 regions uniquely hypomethylated and 5037 uniquely hypermethylated regions specific to motor neurons.

With our optimised deconvolution algorithm we demonstrate it is feasible to detect motor neuron derived DNA present at 1% of of cfDNA

149. WWOX contributes to mitochondrial dysfunction and oxidative stress in ALS

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There is an urgent and unmet need for new and effective therapies for amyotrophic lateral sclerosis (ALS); therefore, understanding the mechanisms underlying neurodegeneration and motor neuron loss is critical. Here, we investigated the role of the WW domain-containing oxidoreductase (WWOX), a protein implicated in neurological and neurodegenerative diseases, such as epilepsy and Alzheimer's disease, in ALS. WWOX plays a crucial role in several cellular functions, such as the regulation of the mitochondrial electron transport chain (mtETC). Given that alterations in the mtETC are widely described in neurodegenerative diseases, including ALS, we hypothesized that alterations in WWOX may contribute to mitochondrial dysfunction in ALS. First, we identified rare and ALS specific variants in WWOX, such as the 261E stop codon mutation, localized in the mitochondrial binding domain of WWOX, by analyzing the Project MinE dataset. Interestingly, the treatment of SH-SY5Y cells with a human recombinant WWOX protein carrying the 261E stop codon mutation decreased cell viability, reduced ATP levels, and increased mitochondrial reactive oxygen species (ROS), which was consistent with our results in human post-mortem motor cortex (mCTX) revealing a significant decrease in mitochondrial ATP synthase of complex V and cytochrome c oxidase of complex IV in ALS. Importantly, these results were also consistent with previous studies indicating alterations in ATP and ROS levels in ALS. Lastly, we demonstrated a significant decrease in WWOX in a large cohort of ALS mCTX. Importantly, knocking down WWOX with a specific siRNA decreased cytosolic and mitochondrial ROS, supporting a link between loss of WWOX and oxidative stress. Together, our findings suggest that the 261E stop codon mutation in WWOX may worsen mitochondrial dysfunction, and loss of WWOX may contribute to oxidative stress in ALS.

150. ZEB1-AS1 IS IMPLICATED IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS THROUGH BETA-CATENIN MODULATION

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Objective: Alterations in transcription are a novel and fundamental cause in the pathogenesis of sporadic Amyotrophic Lateral Sclerosis (sALS) involves alterations in transcription. Indeed, RNA-sequencing analyses from sALS patients tissues highlighted a strong deregulation in long non-coding RNAs (lncRNAs), which thus appear to play a role in the disease. The oncogenic lncRNA ZEB1-AS1 is amongst the top downregulated lncRNAs in peripheral blood mononuclear cells of sALS patients. Interestingly, in cancer-derived cell lines, ZEB1-AS1 belongs to a negative feedback loop regulation with hsa-miR-200c, acting as a molecular sponge for this miRNA. The role of the lncRNA ZEB1-AS1 in sALS pathogenesis is yet to be characterized, and its study could help identifying a possible disease-modifying target.

Methods: the implication of the ZEB1-AS1/ZEB1/hsa-miR-200c/BMI1 pathway was investigated in multiple patients-derived cellular models (patients-derived peripheral blood mononuclear cells and induced pluripotent stem cells-derived neural stem cells) and in the neuroblastoma cell line SH-SY5Y, where its function was inhibited via RNA interference. Molecular techniques such as Real Time PCR, Western Blot and Immunofluorescence were used to assess the pathway dysregulation.

Results: Our results show a dysregulation of a signaling pathway involving ZEB1-AS1/hsa-miR-200c/beta-Catenin in peripheral blood mononuclear cells and in induced pluripotent stem cells-derived neural stem cells from sALS patients. These results were validated in vitro on the cell line SH-SY5Y with silenced expression of ZEB1-AS1. Moreover, we found an increase for ZEB1-AS1 during neural differentiation with an aberrant expression of beta-Catenin, highlighting also its aggregation and possible impact on neurite length. We found a dysregulation of hsa-miR-139 during neural differentiation, and this miRNA is increased following ZEB1-AS1 silencing, suggesting they could also act in a feedback loop which affects beta-Catenin

Conclusions: Our results support and describe the role of ZEB1-AS1 pathway in sALS and specifically in neuronal differentiation, suggesting that it acts in a circuitry with multiple miRNAs leading to an impairment of beta-Catenin signaling and an alteration of the neuronal phenotype.

Translational research

151. 5'Gly-GCC tiRNA levels as a biomarker of disease progression in Amyotrophic Lateral Sclerosis

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Disease progression and survival are variable in Amyotrophic Lateral Sclerosis (ALS), necessitating biomarkers predicting disease progression. Biomarkers can be found through analysis of pathophysiological processes. Here we describe 5'Gly-CC, a tiRNA cleaved from tRNA by Angiogenin (ANG), during the assembly of stress granules. Stress granule homeostasis is dysregulated in ALS and stress granules co-localize with many of the proteins formed by genes that have been linked familial forms of ALS (e.g. TARDBP/TDP-43, FUS, SOD1, ANG). Therefore, we hypothesized stress granule compounds such as tiRNAs could be markers of ALS disease progression. Through RNAseq on longitudinal blood serum samples of 223 ALS patients, 5'Gly-GCC was identified as a promising biomarker, which we validated by qPCR in a cohort of 40 ALS patients and replicated in another 37 patients. Besides a longitudinal increase in serum Gly-GCC in ALS patients, we show that ANG serum levels also increased during disease progression but changes were less pronounced. Concluding, tiRNAs are potential biomarkers for ALS and we plan on further exploring the specificity of this tiRNA for ALS.

152. Biofluid extracellular vesicle extraction and proteomic profiling in ALS

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Extracellular vesicles (EVs) are membrane-bound nanoparticles that are released by cells and carry a range of cargo biomolecules including protein, lipids and RNA. EVs may also be secreted from cells in the central nervous system (CNS), into accessible biofluids such as blood and cerebrospinal fluid (CSF). The use of immunoaffinity capture shows promise to define and separate EVs by cellular origin, with the neuronal lineage marker L1CAM having been employed successfully to identify biomarkers in other neurodegenerative diseases. Our aim is to optimise and validate methods for capture and proteomic profiling of CNS-EVs from blood and CSF for biomarker development in ALS. Here we showed that size exclusion chromatography (SEC) of 0.5-2ml human serum enriches bulk EVs positive for typical EV markers CD63, CD81, CD9, Syntenin-1 and annexin A1 by immunoblotting, and with expected morphology by transmission electron microscopy. We demonstrated that immunocapture of EVs using CD63 or CD81 was effective in reducing apolipoprotein B contamination, but overall efficiency of capture in serum was lower than in cell culture derived EVs. Next, we demonstrated that CD81 was the most highly abundant tetraspanin EV marker in CSF and can be used successfully for immunocapture of EVs from <0.5ml of whole CSF. We then used a multiplex bead-based flow cytometric assay (MACSPlex Exosome Kit) to explore the cellular origins of both CD81 and L1CAM-positive EVs in CSF, plasma and serum. This is an optimised and validated workflow for the enrichment and subsequent proteomic profiling of CNS-cell specific EVs from serum or CSF as a platform for biomarker development in ALS.

153. Biomarkers of neurodegeneration and muscle wasting in amyotrophic lateral sclerosis – a monocentric cross-sectional study

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Amyotrophic laterals sclerosis (ALS) is characterized by degeneration of the upper and lower motor neurons leading to muscle weakness and atrophy. Neurofilaments (Nf), both neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH), are supposed to be promising fluid neurodegenerative biomarkers. Serum creatine kinase (CK), myoglobin (Mb), troponin T (TnT) and creatinine (Crn) are related to muscle damage and also propagated to be predisposing factors on survival and disease progression in ALS. Valuable biomarkers for ALS are particularly needed to ensure early diagnosis, to estimate prognosis and disease progression as well as to monitor disease activity during treatment. Therefore, the aim of this study was to compare these markers within the context of clinical and laboratory characteristics of patients with ALS in a monocentric real-world setting.

56 patients with ALS and its variants (progressive muscle atrophy, PMA; primary lateral sclerosis, PLS) and 19 age- and sex-matched disease controls were included in this retrospective cross-sectional study. Data of Nf, CK, Mb, TnT and Crn were collected and correlated to clinical characteristics such as motor neuron involvement, disease severity and progression assessed by the revised ALS functional rating scale (ALSFRS-R) and its slope (48–ALSFRS-R at visit/disease duration in months).

Nf concentrations were higher in patients with ALS and its variants compared to disease controls. Higher Nf concentrations were also observed in patients with classical ALS compared to patients with PMA. Nf levels strongly correlated with disease duration, total ALSFRS-R score and ALSFRS-R progression slope. Unlike NfL, pNfH was associated with bulbar motor impairment in this cohort.

CK concentrations did not differ between patients with ALS and disease controls, but were lower in ALS patients with bulbar motor involvement and correlated with the ALSFRS-R subscore for respiratory functions. Mb was slightly higher in ALS compared to disease controls. Similarly to CK, Mb was lower in ALS patients with bulbar motor involvement and positively correlated with the ALSFRS-R subscore for respiratory functions. TnT and Crn did not show any relations to the investigated parameters.

In conclusion, Nf showed high diagnostic and prognostic biomarker potential and were superior to the investigated muscle damage markers in ALS and its variants.

154. Brain 2-[18F]FDG-PET as a tool to discriminate Amyotrophic Lateral Sclerosis (ALS) from ALS-mimics

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Introduction. Despite the recent advances in ALS knowledge, a large part of research is still focusing on finding a diagnostic marker to differentiate ALS from its mimicking disorders. Previous studies demonstrated the role of brain 2-[18F]FDG-PET to distinguish ALS patients from healthy controls. One paper reported an 81.5% accuracy using combined brain and spinal cord 2-[18F]FDG-PET to discriminate ALS and ALS-mimics, showing a lower accuracy (65.4%) for brain metabolism alone. Our study aimed at evaluating the role of brain 2-[18F]FDG-PET as a single marker to discriminate ALS from ALS-mimics.

Methods. We included ALS patients referred to the ALS Centre of Turin who underwent brain 2-[18F]FDG-PET at diagnosis between 2009 and 2019, and subjects with ALS-mimicking disorders (including cervical spondylogenic myelopathy, multifocal motor neuropathy, myasthenia gravis). As we considered each voxel as a single feature in our analysis, we employed the Automated Anatomical Labeling Atlas to examine only the whole brain volume in the entire PET scans, thus reducing the number of candidate voxels to 226954. Averaging each voxel for the mean value of the whole brain allowed an intensity normalization at individual level. Since we included 40 ALS mimics, we randomly collected 40 ALS from the 663 of our dataset to calculate Laplacian scores. Subsequently, we extracted features from ALS and ALS-mimics. Finally, we split the dataset (40 ALS and 40 ALS-mimics) in a training set (80%) and a test set (20%) and we used a Support Vector Machine (SVM) approach as classifier. The whole procedure, starting with the random collection of 40 ALS cases and calculation of Laplacian scores, and ending with the SVM classifier training, was randomly repeated 10 times.

155. Cell free miRNA biomarkers for ALS prognostication

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Cell-free biomarkers are greatly needed for precision ALS medicine. microRNAs are endogenous, non-protein coding, small RNAs. We have recently reported the value of circulating microRNA-181 (miR-181) in ALS prognostication (Magen et al., Nature Neuroscience, 2021). Here, we analyzed the effect of plasma miRNAs levels on ALSFRS slope, implementing a mixed model analysis on data from repeated patient visits. We characterized a compound signature of 21 miRNA that predicts the decline rate of ALSFRS-R. Next, we analyzed whether the levels of these miRNAs can also predict mortality risk in two independent cohorts of >200 patients with ALS, each (UK biobank and CReATe cohorts). Higher levels of miR-181, which predicted faster functional decline, also predicted higher mortality risk in both cohorts (hazard ratio=1.4-1.7, $p<0.05$). Interestingly, higher levels of another miRNA, miR-223, predict slower functional decline and lower mortality risks in these cohorts (hazard ratios=0.5-0.7, $p<0.05$). Measurements of these miRNAs in cerebrospinal fluid is also predictive of mortality risk. Since the miRNA prognostic signals are consistent across plasma and cerebrospinal fluid, we suggest that they correspond to pathological processes in the central nervous system and that in combination with protein biomarkers they may improve prognostication and promote ALS clinical development.

156. CHARACTERIZATION OF BLOOD-DERIVED MACROPHAGES OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) FOR THERAPEUTIC AND DIAGNOSTIC APPROACH.

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Motor neurons (MNs) are the cells degenerating in ALS but other cell types surrounding the MNs, especially microglial cells, have been shown to participate to the ongoing neurodegeneration. Our team showed that peripheral macrophages impact disease progression. Replacing mutant macrophages in hSOD1G93A mice by less toxic macrophages slowed disease progression and reduced neuroinflammation in the CNS. Our hypothesis is that ALS monocytes/macrophages could show specific reactive profiles both through their expression of ALS-linked genes and their reaction to MN degeneration. In addition, macrophages at the periphery would be an easier target for therapy than microglia in the CNS. Our aim is therefore to characterize the reactive profiles of blood monocyte and monocyte-derived macrophage populations activated with different stimuli, obtained from ALS patients with familial (FALS) or sporadic (SALS) forms and healthy controls.

To analyze potential dysfunctions of macrophages, we included ALS patients (sporadic and familial cases) as well as asymptomatic individuals carrying ALS mutations and healthy controls. Monocytes from whole blood were differentiated in vitro into macrophages and then activated with pro- or anti-inflammatory stimuli. Immunological responses of monocytes/macrophages were studied at different levels: transcriptome, cells surface marker expression, and secretion profile.

Preliminary results show that, monocytes from controls and all ALS groups were able to differentiate into macrophages and to polarize into pro-inflammatory or anti-inflammatory phenotypes. We then compared FALS, SALS and control derived macrophages, using three different activation conditions. Transcriptome and secretome analyses showed that our samples were clustering in response to the stimuli, but we found groups of patients with different response profiles. Analyses showed that macrophages from patient with C9ORF72 expansion expressed less anti-inflammatory markers on their surface (flow cytometry) and had a pre-activated cytokine secretion profile. Those preliminary results need to be confirmed with a larger patient cohort.

Our results could provide insight into the involvement of macrophages in ALS pathophysiology and reveal target pathways for new therapies and disease biomarkers.

157. Combined spirometric, arterial blood gas analysis and overnight oximetric parameters for the prognosis of patients affected by motor neuron disease.

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INTRODUCTION: The combined use of multiple respiratory test, including spirometry, arterial blood analysis (ABG) and overnight oximetry (OvOx), is highly recommended in motor neuron disease (MND) to monitor the respiratory function of patients and to assess the need of non-invasive mechanical ventilation (NIMV) adaptation. To date there is no definite agreement of the specific individual threshold of NIMV indication and in this study, we propose a composite score whose potential is to simplify the respiratory management and to better stratify prognosis.

MATERIALS AND METHODS: We screened the clinical chart of 471 non-ventilated MND patients referred to the Neuro-rehabilitation Unit of the San Raffaele Institute of Milan (January 2001-December 2019), collecting spirometric, ABG and OvOx parameters. To evaluate the prognostic role of each respiratory measurement, a Univariate Cox-regression for death/tracheostomy was assessed, and the variables associated with survival were selected to design a scoring system. Univariate and multivariate Cox regression analysis were than performed to evaluate the prognostic role of the respiratory score to death/tracheostomy and time to NIMV adaptation. Finally, univariate and multivariate analysis were performed on a control cohort composed of 180 MND patients recruited in the Turin ALS Center.

RESULTS: The overall study population included 450 MND patients. The respiratory variables associated with survival and selected to design a scoring system were: FVC%, ΔFVC%, $HCO3^-$, pCO_2, SBE and MPS. In the final model, assessed trough analysis on the control cohort, we assigned 0 to 3 points for each FVC% interquartile group, an additional point for a ΔFVC% >2 points/month and 1 point whenever the following cut-offs were exceeded: $HCO3^-$ > 26 mmol/L; pCO_2 > 45 mmHg, SBE >2 mmol/L, MPS was <91%. The points of these six variables were then added together to arrive at a final score, with a maximum of total 8 points. Univariate Kaplan-Meier analysis displayed a significant stratification of prognosis according to proposed score values and multivariate Cox regression analysis confirmed the independent effect of the respiratory score on survival of each cohort.

CONCLUSION: FVC, ABG and OvOx parameters provide different and complementary information for the respiratory management and the association of these parameters into a single score might help the neurologist to predict prognosis and to facilitate the respiratory management.

158. Cortical and subcortical grey matter atrophy in Amyotrophic Lateral Sclerosis correlates with measures of disease accumulation independent of disease

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The demand for reliable biomarkers to monitor the progression of Amyotrophic Lateral Sclerosis (ALS) is long considered as highly important and is even more increasing in the light of new potential therapeutic targets (e.g., antisense oligonucleotides).

We therefore conducted this study to investigate the relationship between Magnetic Resonance Imaging (MRI)-derived measures of cortical thickness and subcortical grey matter (GM) volume with D50 disease progression model parameters. We used T1-weighted MRI images of 72 Healthy Controls (HC) and 100 patients with ALS and analyzed them with Surface-based Morphometry for cortical structures and Voxel-based Morphometry for subcortical Region-Of-Interest analyses using the Computational Anatomy Toolbox (CAT12) as implemented in SPM12.

We compared these structural metrics between patients and HC in inter-group contrasts. We also conducted subgroup analyses using parameters of the D50 model, dividing patients by a) phase of disease covered at the time of MRI-scan and b) individual overall disease aggressiveness. Finally, we examined the correlations between GM and D50 model-derived parameters.

Our results showed that ALS-related cortical thinning was mainly located in frontotemporal regions as compared to HC, and that there were smaller subcortical GM volumes in the left hippocampus and amygdala. Comparing patients in different phases of the disease revealed further cortical and subcortical GM atrophy associated with higher disease phases. Regression analyses congruently showed negative correlations between cortical thickness and individual disease covered. However, there were neither differences in cortical thickness and subcortical GM between patients with low and high disease aggressiveness, nor any voxel-wise correlations with this parameter.

By applying the D50 model we were able to identify robust correlations between cortical and subcortical GM atrophy and ALS-related functional disability, but not with disease aggressiveness. This indicates that CT and subcortical GM volume are suitable biomarkers representing individual disease covered/accumulation, independent of disease aggressiveness. This may be important to develop new outcome measures or stratification strategies for upcoming clinical trials and to monitor therapeutic interventions in ALS.

159. Deciphering the role of non-coding variants in the etiology of neurodegenerative diseases (NDDs) by massively parallel reporter assay (MPRA)

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Background/Objectives: In neurodegenerative disorders few patients showed a disease family history indicating that genetics play a role in disease etiology. Missing heritability is still present and could be explained by variants in the non-coding regions of the genome.

This study aimed to perform a high-throughput analysis using MPRA, to analyze several putative regulatory variants simultaneously from WGS data.

Methods: Starting from 870 rare non-coding variants identified through Whole Genome Sequencing (WGS) of 140 patients affected by NDDs, 41 rare non-coding variants of uncertain significance (VUS) located in putative regulatory regions were selected using UCSC-GRCh38/hg38 ENCODE regulation tracks. Among these, 20 were originally found in patient affected by ALS.

A library of 2460 probes (each identified by a different and unique barcode) was designed and cloned in pMPRA vectors upstream of an ORF-sequence and transfected into SHSY5Y cells. After RNA isolation and sequencing, bioinformatics analysis was performed using R version 4.2.0 and mpralm function.

Results: Five variants mapping in the upstream region of ELOVL5, GIGYF2, OMA1, CWF19L1 and NEK1 genes, were found to decrease (OMA1, CWF19L1, NEK1) or increase (ELOVL5, GIGYF2) gene expression (P value ≤ 0,01). The variants in GIGYF2 and NEK1, genes known to be associated with PD and ALS, respectively and involved highly conserved nucleotides, were found each in one patient with consistent clinical phenotype. Variant in OMA1 (a mitochondrial stress response gene) was found in an ALS patient while the variants in CWF19L1 and ELOVL5 (known ataxic genes), in two ataxic patients. All these variants localized in sequences annotated as promoter by UCSC GRCh38/hg38-ENCODE regulation tracks.

Conclusion: Even though the regulatory activity of these variants needs to be confirmed with other functional assays, MPRA was confirmed to be a good tool to screen simultaneously hundreds of non-coding putative pathogenetic variants.

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160. Designing an ALS biomarker panel

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Background: There is a growing need to identify specific biomarkers that facilitate the diagnosis and prognosis of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Currently, the most promising biomarkers in ALS are levels of neurofilament light chain (NfL) (1), circulating miRNA profile (2), circRNAs (3) and biomarkers of inflammation (4). The aim of this study is to develop an ALS biomarker panel based on the identification of accessible blood diagnostic and prognostic molecules.

Methods: 16 target genes and 9 proteins involved in oxidative stress, neuroinflammation, muscle physiology and differentiation, metabolic processes and RNA metabolism were studied by quantitative PCR, western blot and ELISA assays in blood samples. ALS patients were monitored during a follow-up period of 24 months. These levels were related to the main clinical parameters like days since age onset, ALSFRS-r, ALSFRS-r slope, diagnostic delay and others. Statistical analysis was performed using SPSS Statistics version 24 (IBM, Spain).

Results: We found a significant association between increasing COL19A1 gene levels along disease progression and faster evolution of the disease. Additionally, higher COL19A1 levels and a faster progression increased the mortality risk. Regarding circulating miRNA, miR-206 was overexpressed in ALS patients and consistently altered during the course of the disease pathology.

Discussion: Variations in protein levels, mRNAs and circular RNAs involved in ALS indicate the importance of analyzing the proteomic and transcriptomic profile of each patient. The best approach involves the development of a biomarker panel that, considering the patient's genomics, allows for determining their prognosis, supporting clinical diagnosis and exclude other mimetic diseases.

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161. DISCOVERY OF ACI-19278, A POTENTIAL FIRST IN CLASS TDP-43 PET BIOMARKER FOR FTLD-TDP AND ALS

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Inclusions of aggregated transactive response DNA binding protein (TDP-43) in the brain of frontotemporal dementia with TDP-43 (FTLD-TDP) pathology and amyotrophic lateral sclerosis (ALS) patients are a hallmark of these severe diseases with high unmet medical need. TDP-43 is also present as a co-pathology in other diseases such as Alzheimer's and the recently described limbic-predominant age-related TDP-43 encephalopathy (LATE) also manifesting in regions of the brain and spinal cord as disease progresses. Building on our experience to discover tracers for use in positron emission tomography (PET) for other proteins contributing to neurodegenerative diseases such as tau and alpha-synuclein, we aim to identify the first TDP-43 selective small molecule that images the pathology in a living patient. Such a tool is highly sought after as a biomarker to better diagnose disease and stratify patients for enrolment in clinical trials. To date, several chemical series have been identified from screening of our proprietary brain-penetrant and beta-sheet binding Morphomer® small molecule library. Using in house established radiobinding assays, involving brain derived aggregated TDP-43, and autoradiography, on brain sections from patients with TDP-43 proteinopathies, several compounds have been selected for pharmacokinetics evaluation in non-human primates. Among these, ACI-19278 demonstrates an affinity in the low nanomolar range for TDP-43 aggregates by radiobinding assay and differentiates FTLD-TDP type A pathology from control tissue by classical autoradiography on brain sections. The direct binding was confirmed by high resolution autoradiography, where colocalization of the compound with phospho-TDP-43 immunoreactive inclusions was observed. ACI-19278 was assessed for selectivity over amyloid beta and a-synuclein in binding assays using extracts derived from brains with Alzheimer's and Parkinson's disease, respectively. In a pharmacokinetic study administering [18F]-ACI-19278 to a non-human primate, the compound demonstrated favourable brain uptake and washout, profiles compatible with the required characteristics of a developable PET tracer. Taken together, our discovery of ACI-19278, a small molecule that binds sufficiently to TDP-43 in brain sections of patients with FTLD, provides the first evidence towards a transformative biomarker to be used to image TDP-43 in patients living with TDP-43 proteinopathies.

162. Evaluating the characteristics of High Frequency Evoked Responses as a potential biomarker for sensorimotor dysfunction in ALS: A Preliminary Study

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Background: Previous studies[1][2] indicate that the low amplitude high frequency (LHW) responses recorded at cervical spinal levels might be generated by long sensory tracts. The characteristics of LHW response change in neurological conditions affecting the spinal cord and during voluntary contraction. Here we investigate the feasibility of non-invasive recording of LHW oscillations. This will enable the evaluation of LHW-based biomarkers related to sensorimotor dysfunction in ALS for the first time.

Objective: The overarching objective is to investigate the feasibility of evoked LHW components recorded at the cervical spinal level in response to the median nerve stimulation as a non-invasive biomarker.

Methodology: Recruitment of healthy young adults is ongoing, with data from 5

participants collected. The non-invasive recording was performed by surface electrodes placed on the neck of the participant at C6 vertebral level in accordance with the ring electrode placement system and electrophysiological signals were recorded at the sampling rate of 8kHz. A total of 1600 evoked responses (trials) were recorded in response to the median nerve (MN) stimulation at wrist (1.5 X Motor Threshold, 2Hz). The recorded signals were pre-processed to remove noise and artifacts and were bandpass filtered between 350-2000Hz. The LHW evoked responses were obtained by averaging the resulting signals across all trials.

Results: The LHW responses were observed in response to the MN stimulation. The duration of the detected LHW responses was 13.4 ± 3.24 (mean ± SD, ms). The recorded LHW peak amplitude and latency were 0.9 ± 0.4 (mean ± SD, uV) and 11.7 ± 1.2 (mean ± SD, ms). The LHW onset latency was 6.1 ± 1.4 (mean ± SD, ms).

Discussion: The preliminary results on young healthy participants indicate that LHW responses can be recorded non-invasively at the cervical level and their characteristics were comparable to the previous literature where the responses were recorded invasively at the epidural level. This study demonstrates the feasibility of LHW evaluation in ALS for potential sensorimotor biomarkers.

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163. Evaluation of arterial blood gas parameters as prognostic markers in amyotrophic lateral sclerosis

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Background: Forced vital capacity (FVC) remains difficult to determine for some patient suffering from amyotrophic lateral sclerosis (ALS) due to the rapid progression of the disease. Arterial blood gas (ABG) parameters could represent a valuable alternative. The aim of this study was therefore to evaluate the correlation between ABG parameters and FVC, along with the prognostic ability of ABG parameters in a large cohort of ALS patients.

Method: 302 ALS patients with FVC and ABG parameters available at diagnostic were included. Correlations between ABG parameters and FVC were evaluated. Cox-regression was then carried out to determine association of each parameter (ABG and clinical data) with the survival. Finally, Receiver Operating Curves (ROC) were built to predict the survival of ALS.

Results: HCO3 - , pO2 , pCO2 , base excess (BE), oxygen saturation and oxyhemoglobin were significantly correlated with FVC for both patients with spinal or bulbar onset. Univariate Cox regression showed that HCO3 - and BE were associated with survival, but only in spinal forms. ABG parameters predicted the survival of ALS with similar performance to FVC, HCO3 - being the parameter with the highest area under the curve.

Conclusion: Our results suggest that there is an interest to conducting a longitudinal evaluation throughout disease progression to confirm the equal performances between FVC and ABG. This study highlights the benefits of performing ABG analysis that could be used as an interesting alternative to FVC when spirometry cannot be performed.

164. Extracellular Vesicles as Potential Biomarkers in Amyotrophic Lateral Sclerosis

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The diagnosis of amyotrophic lateral sclerosis (ALS) remains challenging because the disease progresses slowly and is often accompanied by other neurologic comorbidities. In this review, we compiled recent studies aimed at identifying extracellular vesicles (EVs) as potential biomarkers for ALS focusing on their size, number, and EV-associated protein, lipid, and nucleic acid cargo, in blood and CSF from ALS patients.

EVs are small membrane-bound vesicles released by cells into the extracellular space whose main function is to exchange information with other cells. Their molecular information changes greatly depending on the type of parent cell and pathophysiological conditions, making them potential prognostic and diagnostic ALS biomarkers. ALS has been associated with disorders of vesicle-mediated transport and autophagy, as well as the cell-autonomous onset of disease in glutamatergic neurons. Because EVs can cross the blood-brain barrier and are peripherally available, the use of EVs may be key to accessing pathologically relevant tissues in neurodegenerative diseases, including ALS. Several proteins, as well as CUEDC2 mRNA and miR-124-3p, have been suggested as potential ALS biomarkers in CSF-derived EVs. In blood-derived EVs, studies described decreased levels of HSP90 and PPPIA and increased levels of CORO1A, as well as elevated levels of toxic SOD1, TDP-43, phospho-TDP-43, and FUS proteins in large EVs (LEVs). IL-6, a neuroinflammation marker, was detected at elevated levels in astrocyte EVs isolated from plasma. In addition to protein cargo, a specific common signature of 15 differentially expressed mRNAs was detected in the serum of ALS patients, which were present in both small extracellular vesicles (SEVs) and LEVs. In addition, plasma-derived LEVs contained high levels of lipids but low levels of the aromatic amino acid phenylalanine, a potential biomarker for ALS diagnosis. Examination of the miRNA content of blood-derived EVs revealed that miR-4454 and miR-127-3p were deregulated in several studies, albeit in opposite directions. Finally, five miRNAs were proposed as the miRNA fingerprint: miR-151a-5p and miR-146a-5p which were upregulated, and miR-4454, miR-10b-5p, and miR-29b-3p which were downregulated. Although there are still some technical challenges we believe that in the near future, EVs may become clinically useful biomarkers and a new source of information about disease pathogenesis.

165. First evidence of altered Gas6-Axl signaling and correlation between sAXL blood levels and clinical decline in ALS

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Axl belongs to TAM tyrosine kinase receptor family and is involved in neuronal survival, myelination and regulation of immune responses. Up-regulation of Axl was reported in disease-associated microglia in Alzheimer's disease (AD), in multiple sclerosis (MS) and, more recently, also in a mouse model of TDP43 proteinopathy. Gas6 (Axl endogenous ligand) is important to modulate the extent of neuroinflammation and remyelination in the Central and Peripheral nervous system. One mechanism of regulation of Gas6/Axl pathway is the cleavage of Axl extracellular domain by sheddases ADAM10/17, which are upregulated in proinflammatory conditions. Axl shedding leads to the release of a soluble ectodomain (sAxl) or the Gas6-Axl complex and failure of the membrane-bound Axl portion to transduce intracellular signals.

Given the role played by Gas6-Axl pathway in neuronal survival and neuroinflammatory responses, in this work we investigated, for the first time, whether this pathway is implicated in ALS.

We analyzed Gas6-Axl pathway in mutant SOD1 and TDP43 animal models of ALS. In particular, we assessed: i) Gas6 and Axl distribution and expression levels (by RNAscope matched with IHC); ii) phospho-Axl (pAxl) and ADAM sheddase activity; iii) the correlation between alterations of Gas6-Axl pathway and changes in the levels of sAxl measured in the serum along the disease progression in animal models, as well as in a small cohort of ALS patients.

In non-transgenic (NTg) animals, Axl is localized in ventral horn (VH) spinal cord motor neurons (MNs). On the contrary, in TG animals, Axl is downregulated or not detectable (both at protein and mRNA level) in MNs whereas it is upregulated in reactive astrocytes and microglia. Gas6 is detectable only in MNs and axons in NTg whereas in symptomatic TG animals it is present only in few surviving MNs and axons and it is barely detectable in few glial cells despite extensive astro- and micro-gliosis. In parallel, pAxl is downregulated in spinal cord MNs of symptomatic TG animals whereas ADAM10/17 sheddase activity is increased. Interestingly, we detected significantly increased levels of AXL ectodomain (sAXL) in the serum of ALS patients, compared to healthy controls.

Overall, we evidenced for the first time a defective Gas6-Axl pathway in ALS, highlighting that these alterations could account not only for the engagement of reactive gliosis, but also for defective neurotrophic signaling and MN demise.

166. Hsa_circ_0060762 and CSE1L are potential peripheral blood biomarkers and therapeutic targets for ALS

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive adult-onset neurodegenerative disease that is often diagnosed with a delay due to initial non-specific symptoms. Therefore, reliable and easy-to obtain biomarkers are in desperate need for earlier and more accurate diagnostics. Circular RNAs (circRNAs) have been already proposed as potential biomarkers for several neurodegenerative diseases. In this study, we further investigated the usefulness of circRNAs as potential biomarkers for ALS. We first performed a microarray analysis of circRNAs on peripheral blood mononuclear cells of a subset of ALS patients and controls. Among the differently expressed circRNA by microarray analysis, we selected only the ones with a host gene that harbors the highest level of conservation and genetic constraints. This selection was based under the hypothesis that genes under selective pressure and genetic constraints could have a major role in determining a trait or disease. Then we performed a linear regression between ALS cases and controls using each circRNA as a predictor variable. With an FDR threshold of 0.1, only six circRNAs passed the filtering and merely one of them remained statistically significant after Bonferroni correction: hsa_circ_0060762 and its host gene CSE1L. Finally, we observed a significant reduction in expression levels between larger sets of patients and healthy controls for both hsa_circ_0060762 and CSE1L. CSE1L is a member of the importin β family and mediates inhibition of TDP-43 aggregation. Inhibition of the importin α/β pathway cause cytosolic retention of TDP-43 and promote protein oligomerisation and aggregation, the central pathogenicity in ALS. In addition, receiver operating characteristics curve analysis showed diagnostic potential for CSE1L and hsa_circ_0060762. Hsa_circ_0060762 and CSE1L thus represent novel potential peripheral blood biomarkers and therapeutic targets for ALS as well as open possibilities for further investigations of their role in ALS pathogenesis.

167. Hypothalamic correlates of metabolism and cognition in ALS

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Introduction

Dysregulation of energy homeostasis and cognitive impairments are important non-motor symptoms in amyotrophic lateral sclerosis (ALS), with a negative effect on survival and quality of life. The hypothalamus plays an essential role in maintaining energy balance trough regulation of energy intake and expenditure and, as part of the limbic system, in regulating cognition. Therefore, we evaluated hypothalamic neurodegeneration in ALS and explored the relationship between hypothalamic subregions and changes in energy balance, cognitive deficits, and disease progression.

Methods

We included 566 patients with ALS and 365 controls for this cross-sectional and longitudinal case control study. Information on body mass index (BMI), weight loss, C9orf72-carriership and deficits in cognitive domains, determined using the Edinburgh cognitive and behavioural ALS screen (ECAS), was collected. T1-weighted magnetic resonance imaging was performed in all participants. The hypothalamus and its subregions were segmented, and volumes were calculated. Linear (mixed) models, adjusted for age, sex and total intracranial volume, were used to compare hypothalamic volumes between groups and to analyse associations with metabolism and cognition. Permutation-based corrections for multiple hypothesis testing were applied for all analyses to control the family wise error rate.

Results

Compared with controls, the volume of the anterior superior subregion of the hypothalamus was smaller in patients with ALS ($p = 0.01$). After correction for multiple testing, there was no statistically significant effect of ALS on other subregions or effect of C9orf72-carriership. Weight loss and memory impairments were associated with a smaller posterior hypothalamus (both $p < 0.01$), the volume of this subregion decreased faster over time in ALS patients than in controls ($p = 0.01$) and was correlated with a shorter survival ($p = 0.03$).

Discussion

In this largest hypothalamic study in ALS to date, we demonstrated that the anterior superior subregion of the hypothalamus is affected in ALS and posterior pathology is associated with weight loss, memory dysfunction, deterioration over time and survival. These results suggest a role of the hypothalamus in dysregulation of energy homeostasis and cognitive impairments in ALS and may have important implications for the identification of new treatment targets for this devastating disease.

168. Identification and validation of a protein biomarker signature from tear fluid in patients with Amyotrophic Lateral Sclerosis (ALS)

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Background: The diagnosis of ALS is based on the clinical presentation and remains challenging. The aim of this study was to evaluate the potential of tear fluid (TF) as a biomarker source to identify patients with ALS. TF is easily accessible and has a spatial relation to the CNS. Previous studies showed significantly increased soluble alpha-synuclein levels in TF from patients with Parkinson's disease.

Methods: In a discovery approach, unstimulated TF from 49 patients with probable or definite ALS according to the revised El Escorial criteria and 54 age-correlated controls (CTR) without evidence of neurodegenerative disease were obtained using Schirmer test strips. Subsequently, the extracted protein lysates were analyzed by data independent acquisition mass spectrometry (DIA-MS). Furthermore, machine learning models were trained to stratify ALS and CTR samples. All models were 10-fold cross-validated and 500x bootstrapped. A panel of proteins was selected based on feature importance of predictive models computed by receiver operating characteristic (ROC) and area under the ROC curve (AUROC) analyses. Subsequently, the protein signature was validated using Western blot in an independent cohort, i.e., unstimulated TF from 52 patients and 52 CTR.

Results: Of the 876 proteins identified with DIA-MS in the DC, biostatistical analyses showed a significantly higher abundance in ALS for 12 proteins and significantly lower abundance for 94 proteins. We observed an AUROC of 66.1% for the selected protein signature, which included Haptoglobin (HP), Antithrombin-III (SERPINC1), F-actin-capping protein subunit alpha-2 (CAPZA2), My-crystallin (CRYM), Aldehyde dehydrogenase family 16 member A1 (ALDH16A1), Phosphofructokinase. All of these proteins showed significantly decreased abundancies in the TF of ALS patients of the DC. In the WB analysis of the validation cohort, a decreased abundance of HP in ALS patients could be validated, while SERPINC1, CAPZA2, CRYM and ALDH16A1 showed trends for lower abundancy in ALS.

Conclusion: Using TF samples from ALS patients and CTR, we could identify differentially regulated proteins and select a panel of 6 candidates for validation by Western blot. Of those, Haptoglobin could be validated in an independent cohort suggesting that it may contribute to a biomarker signature of ALS in TF. Additional studies with larger cohorts will be needed to evaluate the potential of TF proteins for the diagnosis of ALS.

169. Identification of a disease signature for premotor and early ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is the most common motoneuron disease with a long diagnostic delay. Because of the rapid and fatal progression of ALS, early diagnosis is crucial to start therapy early and allow for inclusion in clinical trials. About 10% of ALS patients have a genetic cause. Genetic testing of their family members can identify subjects, who carry the mutation, but have not yet developed symptoms of the disease, so-called “pre-symptomatic gene mutation carriers” (PGMC). We aim to identify early ALS biomarkers already present in PGMC.

Method: For this study, 10 different countries join forces (Germany, France, Switzerland, Turkey, Slovakia, Israel, Sweden, Poland, Australia and USA). PGMC, control subjects and patients with suspicion for ALS (n=110 per group) will be recruited and longitudinally assessed over one year. Assessments include a medical history questionnaire, neurological examination, and the collection of biological samples (serum, plasma, urine, tear fluid, and CSF). Proteomic and metabolomic profiles will be analyzed by mass spectrometry and targeted immunoassays.

Results: The “premodiALS”-study secured funding within the JPND-call 2021 and received ethical approval. Recruitment started in Q1/2023.

Discussion: A clinical and molecular ALS fingerprint would improve the timeliness and accuracy of diagnosis of ALS and ultimately lead to more effective treatment strategies.

170. Identification of non-coding RNA that correlate with prognosis and progression in the serum of people with amyotrophic lateral sclerosis.

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Objective markers which can not only aid in diagnosis but be used to predict prognosis and track progression are critically needed for amyotrophic lateral sclerosis to not only improve care, but also help support clinical trials to succeed. We have been interested in the use of the small molecular class non-coding RNA (ncRNA) as potential biomarkers for the disease due to their stability and ease of detection in biofluids such as serum and plasma from blood as well as their role in the pathology of the disease. Our previous work has focused on identifying ncRNA candidates in serum to help with diagnosis, but our more recent work is focused on investigating our hypothesis that ncRNA can also predict and track how the disease progresses, which will provide insight into the mechanisms of pathology.

To this end, we undertook small RNA-sequencing on serum samples collected longitudinally in Sheffield, United Kingdom from 75 people with ALS over the course of their disease. Using a negative binomial mixed effects model to correct for variation in sampling, progression, and individuals, limiting to the 105 ncRNA that showed at least 25 reads on average across all samples, we identified a total of 29 different ncRNA that were significantly altered with time and/or progression with 30 predicted to be markers of prognosis using a log-rank test. Using RT-qPCR, we have to date validated five ncRNA including the microRNA (miR-21-5p, miR-23a-5p, miR-30e-5p), piwi-interacting RNA (piR-33151), and 5' tRNA fragments (TRA-AGC6) that change over time with disease progression independent of age. Interestingly, for three of these ncRNA (miR-21-5p, piR-33151, TRA-AGC6), there is evidence demonstrating that they change over time differently based on the speed of the disease progression, suggesting that these may be signalling differences in the molecular pathology of the disease, with two predictive of prognosis at the point of first sampling (miR-23a-5p, TRA-AGC6). Furthermore, we have also detected the previously identified miR-181a-5p as a potential stable marker for prognosis.

As such, this work provides promise that these ncRNA could not only be predictive of prognosis and trackers of progression in addition to aiding in diagnosis, but that they can highlight potential pathological mechanisms that underlie the disease.

171. Implication of central central nervous systems barriers impairment in amyotrophic lateral sclerosis patients : a sex-related difference

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Central nervous systems (CNS) barriers impairment has been reported in Amyotrophic lateral sclerosis (ALS) since a decade, highlighting its potential interest in ALS, via its putative implication in pathogenesis mechanism, as a biomarker of disease evolution and finally as required parameter to work on drug development. In this context, we aim to shed light about its implication in the disease, with the determination of albumin quotient (QAlb) at the time of the diagnostic of ALS in a large cohort of patients.

307 patients from the universitary hospital of Tours were included in this single centre and retrospective study. According to the previously reported sex-related differences in QAlb levels in healthy controls and patients, male and female subjects were analysed separately. Nighty-two patients (30%) had an elevation of QAlb according to age-related upper reference limit. This percentage was higher in male (43%) than in female (15%). Concerning the continuous values of this parameter, male had as expected significant higher levels than female (median QAlb in male = 0.76 %, quartiles =0.59-1.02 %) (median in female= 0.58 %, quartiles =0.47-0.74%, p <0.001). Interestingly, QAlb was not associated with age of onset, age at sampling or diagnostic delay, neither in male or female patients. However, we found an association with ALSFRS-r at diagnostic but this was significant only in male. QAlb levels was not different between patients with genetic forms of ALS and the other patients. Then , we performed a survival analysis and Kaplan-Meier curves made with two groups according to the median value of QAlb in male and female displayed association with survival in male only. Using multivariate cox proportional hazard, QAlb remains significantly associated with the survival in male patients (HR = 2.3, 95% CI = 1.2-4.3, p=0.009).

To conclude, we found that CNS barrier integrity is altered in many male ALS patients and that QAlb levels is associated with survival in this specific population. A Longitudinal evaluation of this marker along the evolution of ALS, in combination with inflammatory biomarkers could give insight about the implication of CNS barrier alteration in the pathogenesis of the disease. Our results also suggest the interest to decipher in more detail the sex-related difference in barrier leakage. This gender difference might guide the development of new drugs and help personalise the treatment of ALS.

172. Investigating the suitability of urinary biochemical analytes to assess nutritional state in MND: a pilot study

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A decline in nutritional state is negatively associated with functional change and prognosis in MND. Current approaches to nutritional assessment in the clinic are far from optimal, with measurements of percentage weight change from diagnosis recommended in the ESPEN guidelines. We aimed to investigate the suitability of biochemical analytes measured from 24-hour (24h) urinary collections to assess nutritional state in MND.

We conducted an observational, prospective, longitudinal cohort study to compare self-reported 24h dietary intake against 24h urinary biochemical analytes for potassium, sodium and protein intake. Bivariate correlation analysis between nutrient intake and urinary analytes (sodium, potassium, urea and total urinary nitrogen (TUN)) was conducted at baseline. Longitudinal analysis using a repeated measures test was conducted at four intervals over nine-months to assess changes in urinary analyte concentrations. Changes in urinary analyte concentrations were compared to dietary intake and existing assessments of nutritional state using percentage weight change from baseline.

Twenty-two people living with MND were included in baseline analysis. 24h urinary potassium and TUN demonstrated a moderate positive correlation with 24h potassium ($r = 0.456$, $p = 0.033$) and protein ($r = 0.482$, $p = 0.023$) intake at baseline (month 0). Thirteen participants were included in longitudinal analysis. 24h urinary potassium and TUN continued to demonstrate moderate positive correlations with potassium ($r = 0.596$, $p = 0.006$) and protein ($r = 0.509$, $p = 0.022$) intake at the second study visit (month three), but not at subsequent visits (months six and nine). The mean concentration of 24h urinary potassium was observed to decrease sequentially at each time point, but this did not reach significance ($p = 0.060$). There was no statistical significant difference between any time points for weight change or 24h intake.

Longitudinal changes in reported 24h potassium and protein intake did not reflect changes in 24h urinary potassium and TUN after three months. The reason for the loss of significance between intake and excretion is unknown. Errors in reported dietary intake, sequestration of nutrients, hypermetabolism, or the completeness of 24h urinary collections are potential factors that need to be explored to better understand this relationship. The suitability of urinary biochemical analytes to assess nutritional state in MND needs further examination.

173. Lys-acetylated PPIA as a possible translational biomarker of ALS

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TDP-43 pathology is a prevalent neuropathological hallmark found in up to 97% of ALS patients. Alterations in TDP-43 trafficking lead to cytoplasmic mislocalization, aggregation, fragmentation and hyper-phosphorylation of TDP-43. We demonstrated that peptidyl-prolyl cis-trans isomerase A (PPIA), also known as cyclophilin A, a foldase and molecular chaperone, is an interacting partner of TDP-43 and regulates its trafficking. In absence of PPIA, TDP-43 pathology is induced and disease progression exacerbated in the SOD1G93A mouse model of ALS. Additionally, we found that the interaction between PPIA and TDP-43 depends on PPIA acetylation at lysine residue 125 (acetyl-K125-PPIA). We detected low acetyl-PPIA levels and impaired PPIA/TDP-43 interaction in peripheral blood mononuclear cells (PBMCs) of sporadic ALS patients, which also display TDP-43 pathology. These findings suggest that acetyl-K125-PPIA may be a promising biomarker of ALS. However, there is no simple and robust assay to measure acetyl-K125-PPIA levels. To address this issue, we developed two methods to analyze and quantify acetyl-K125-PPIA in human and mouse samples. The first method employs liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode that we tested with lysates from human cell lines and patient PBMCs. This method allowed us to identify and quantify the PPIA acetylated and non-acetylated K125-containing peptides generated by tryptic digestion and a common peptide to quantify the total PPIA content. To develop a method more suitable to a clinical setting, we generated a polyclonal antibody that recognizes human and mouse acetyl-K125-PPIA. For rabbit immunization, we used a chemically synthetized dendron-like tetravalent poly-lysine core conjugated to acetylated PPIA peptides around K125. We obtained a highly specific antibody for the acetylated form of PPIA through a two-step affinity purification process of the antiserum. We tested the antibody by Jess Simple Western® with lysates from human cell lines, PBMCs of patients and spinal cord tissues of ALS mouse models. We are conducting longitudinal analyses of PBMCs from ALS patients and healthy controls using these two methods. In conclusion, we developed two reliable approaches to analyze and quantify acetyl-K125-PPIA content in a variety of biological samples paving the way for validating this protein as a promising translational biomarker.

174. Macrophage inclusions in cerebrospinal fluid following treatment initiation with antisense oligonucleotide therapies in motor neuron diseases

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5q-associated spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) are aetiologically different motor neuron diseases sharing degeneration of lower motor neurons. The antisense oligonucleotide (ASO) nusinersen, the first intrathecally administered gene therapy for SMA, was shown to be effective in a large proportion of patients with SMA and was approved in 2017 by the European Medicine Agency. The ASO tofersen was developed for patients with superoxide dismutase mutation-associated ALS (SOD1-ALS) to reduce toxic SOD1 protein level. The VALOR study, a phase III trial to prove efficacy of tofersen, did not meet the primary endpoint, however is currently being further evaluated in an open-label extension phase and available for patients with SOD1-ALS in an early access program.

In this report a case series of CSF cytology findings in patients with SMA and ALS revealing comparable unspecified macrophage inclusions following treatment initiation with the ASOs nusinersen and tofersen will be presented. In two patients, macrophages with multiple eosinophilic and basophilic inclusions within intracytoplasmic vacuoles were detected during ASO treatment, while these findings were not observed before treatment initiation. Furthermore, a few macrophages with inclusions appeared with nearby located lymphocytes indicating lymphocytic interaction. The composition of these inclusions is still unknown. However, it can be hypothesized that the observation of macrophage inclusions may reflect activation of the innate immune system due to intrathecal ASO treatment. To date, nusinersen and tofersen were shown to be well tolerated, nevertheless the reported findings need to be further investigated as these treatments are long-term therapies with unknown benefit-risk ratios after long-term use.

175. Multiparametric MRI texture and diffusion tensor imaging of the corpus callosum and cortico-spinal tract as a potential biomarker for PLS

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Background:

In patients with primary lateral sclerosis (PLS), multiparametric MRI texture and diffusor tensor imaging (DTI) recent studies showed alterations in the cortico-efferent tracts and in the corpus callosum (CC), especially in CC area III (motor area). We aimed to evaluate whether these alterations correlate with disease severity and thus could be used as a biomarker.

Methods:

This cross-sectional study includes 34 patients with primary lateral sclerosis and 34 age- and sex-matched controls who underwent an MRI scan with standardized research sequences. We analyzed fractional anisotropy (FA) of the cortico-spinal tract and FA, volume (normalized on entire CC volume in the same patient), homogeneity, and entropy of the CC area III. We compared these parameters between patients with PLS and controls and correlated them with the extent of clinical affection, defined as the number of regions (lower extremities, upper extremities, bulbar) with clinical signs of upper motor neuron degeneration as well as the ALSFRS-R sum score.

Results:

Patients had a median ALSFRS-R sum score of 42 points (IQR 38 - 43), a median disease duration of 2.4 years (IQR 1.3 - 6.2), and a median disease progression rate (ΔFRS) of -0.21 pt/m (IQR -0.08 to -0.33). Patients with PLS (mean age 60.9 ±10.0 years) showed significant alterations compared to matched controls (mean age 60.9 ±9.9 years) in all parameters investigated: volume CC area III $p=0.005$; FA of the cortico-spinal tract and FA, homogeneity, and entropy of the CC area III each $p<0.001$. Combining the alterations observed in FA of the cortico-spinal tract and FA, homogeneity, and entropy of the CC area III in a single parameter enabled us to perfectly separate controls from PLS. Furthermore, the combined parameter separated patients with PLS according to the number of regions affected (1, 2, or 3 regions) and correlated with the ALSFRS-R sum score ($r=0.471$).

Conclusion:

A combination of multiparametric MRI texture and DTI analysis facilitates the separation of patients with PLS from controls and correlates with disease severity, indicating potential use as a diagnostic and prognostic biomarker. Longitudinal studies are needed to evaluate the utility as a marker for disease progression in individual patients.

176. Plasma Neurofilament light chain levels associate with nutritional status and disease progression in Amyotrophic Lateral Sclerosis

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Background and aim: The majority of Amyotrophic Lateral Sclerosis (ALS) patients develop some degree of malnutrition during the course of the disease; it is observed in 16%-55% of patients at diagnosis [1]. However, there is not much evidence on the value of using malnutrition tools in predicting the prognosis of these patients. Molecular biomarkers could be helpful in this regard. The potentiality of Neurofilament light chain (NF-L) as a marker of disease severity or rate of disease progression in ALS has been reported [3]. However, its relationship with the malnutritional status or with the metabolic alterations in ALS that are considered major prognostic factors has not been described. The aim of this study was to correlate the NF-L levels with the nutritional status and progression rate in an ALS cohort.

Methods: Demographic and clinical data, including BMI, weight loss percentage and rating of ALSFRS-R were collected for 90 ALS patients (mean [SD] age, 60.9 [12.6] years; 48 female). Disease progression rate was calculated using the ALSFRS-R scale. The Global Leadership Initiative on Malnutrition was used for the assessment of the nutrition status. Blood samples were collected from all participants and pNF-L quantification was performed using Simoa technology.

Results: There was a strong correlation of pNF-L concentrations with the disease progression rate ($p < 0.0001$, $r = 0.425$), with BMI (the $p=0.0059$, $r=-0.299$), and with the weight loss percentage ($p=0.0107$, $r=0.2773$). A sub-group analysis of ALS patients showed significantly higher levels of NF-L in faster progressor patients. Furthermore, higher levels of pNF-L were also found in underweight patients (BMI <18.5) compared to patients with normal weight and in patients with the highest weight loss percentage.

Conclusions: Our finding of elevated plasma levels of NF-L in patients with fast progression compared to slow progressors are consistent with previous data from literature. However, our study adds insights into the role of the nutritional status of ALS patient and its direct effect on the motor neuron degeneration and rate of disease progression. Further studies, including larger number of patients and testing together other fluid biomarkers, are needed to better explain the role of metabolic alterations in the ALS progression and severity.

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177. Precision Medicine for Amyotrophic Lateral Sclerosis: Combinatorial Analysis of Patient Genomes

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Amyotrophic lateral sclerosis (ALS) is a complex, progressive neurodegenerative disease. As the currently approved therapies for the treatment with ALS are scarce and have limited clinical benefits, there is an urgent need to find new targets for therapeutic interventions. GWAS and other approaches have identified several disease-associated genes, but these findings have not translated into progress in clinical trials. This probably reflects the fact that GWAS is limited to identifying single variants with large effect sizes in a population, while the key to understanding complex diseases such as ALS that are influenced by multiple genetic loci, epidemiological and/or environmental factors is to find combinations of these disease associated factors that distinguish one patient subgroup from another.

The PrecisionLife (PL) platform utilises a hypothesis-free method for the detection of combinations of features that together are strongly associated with variations in disease risk, progression rates and other clinical phenotypes often observed in ALS patient subgroups.

Using patient datasets accessed as part of a collaboration with King's College, London and the UK's Motor Neuron Disease Association, PL has performed a detailed genetic stratification of ALS patients, which has uncovered multiple genetic associations particularly linked to sporadic ALS. We identified 33 novel targets, including ones associated with fast disease progression and progressive muscular atrophy phenotypes. Among this panel we also found targets potentially linked to existing drugs, e.g., riluzole and retigabine. For each of the targets, the PL analysis has generated a set of companion genetic biomarkers to facilitate identifying patients predicted to respond to modulation of the protein. We have selected the highest scoring, most druggable targets to be validated in ALS-relevant, human iPSC-neuronal models including patient derived cells, followed by in vivo studies in animal models (zebrafish and mouse).

The results demonstrate that the PrecisionLife combinatorial analysis is uniquely able to stratify heterogenous patient populations with complex disease pathologies. We can use these insights to identify more effective therapeutic strategies and accompanying biomarker sets to match them to the patient subgroups that are most likely to demonstrate benefit in downstream clinical trials.

178. Predictors of SOD1-ALS progression: role of sex and cancer from two population-based registries

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Background: The ALS phenotype associated with SOD1 mutations (SOD1-ALS) has distinct features, although some variants are associated with specific disease trajectories. There are few data on the relationship between ALS and cancer prevalence, especially among SOD1 carriers, for whom SOD1 might play a role in tumor development and growth. Our study evaluated whether SOD1-ALS exhibit distinct features compared with the general ALS population and whether comorbidities, particularly neoplastic diseases, influence ALS progression in these patients.

Methods: This is a retrospective observational study of a cohort of Italian patients with SOD1-ALS, collected from the Emilia Romagna and Piedmont and Aosta Valley registries. We analyzed clinical features and genotype-phenotype correlations, considering the predictive impact of each mutation and focusing on the role of sex and cancer on disease progression.

Results: Among 2204 genotyped ALS patients, 2.5% carried SOD1 mutations. This subgroup includes 25 males, with a M:F ratio of 0.83. SOD1-ALS patients were significantly younger than the other patients (wm-ALS) with a more frequent family history of ALS or FTD. No differences were found regarding sex, weight loss at diagnosis, diagnostic delay, presence of FTD or parkinsonism. SOD1-ALS had a slower progression rate at diagnosis and mean tracheostomy free-survival from the onset, although with high variability: a survival shorter than one year was found for L39V, G42S, G73S, and D91N mutations. SOD1-ALS phenotypic spectrum runs from flail leg, to the rare UMNp or bulbar phenotypes. Among SOD1-ALS, we observed aggressive disease in male patients, with faster progression and lower FVC at diagnosis and shorter time to NIV. Multivariate analysis of survival showed that a history of neoplasms, limb onset with respect to bulbar region, and mutations localized in other exons than 2, were independently associated with better survival. Finally, patients with an oncologic history had a trend toward a slower rate of progression and a milder monthly decline in FVC.

Interpretation: SOD1-ALS group could present some peculiarities in relation to cancer presence and sex distribution, along with the specific impact of mutations on SOD1 function. Considering current therapies, the study of phenotypic heterogeneity associated with SOD1-ALS is even more important as it could represent a potential modulator of the effectiveness of different therapeutic strategies.

179. Prognostic prediction models in motor neuron disease: a systematic review

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Background:

The variability of disease progression in motor neuron disease (MND) makes predicting disease trajectory challenging. Clinically, this impacts our ability to anticipate the timing of care interventions that have an optimal initiation window. For people with MND, the lack of personalised prognostic information can negatively impact emotional wellbeing, hampering the ability to plan for the future with certainty. In clinical trials, clinicians and industry partners are less able to stratify patients most likely to benefit from novel therapeutics.

Prognostic prediction models estimate an individual's risk, or probability, of a future health outcome, such as need for therapeutic intervention or survival time. There is an increasing body of literature proposing such models in MND, however little is known about the clinical utility, methodological quality, or reporting standards of these studies.

Methods:

We conducted a systematic review of MEDLINE and Embase databases for articles published from database inception that describe the development and/or validation of a prognostic prediction model in MND. Outcomes of our review were the risk of bias, determined using the Prediction model Risk Of Bias ASessment Tool (PROBAST), and standard of reporting, assessed using the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) guidance. We also provide a narrative description of the current integration of prognostic prediction models in MND clinical care and trials.

Results:

9551 records were screened, with 51 publications included. Data extraction and analysis detailing and assessing the predicted outcome(s), risk and source(s) of bias, standard of reporting, and clinical utility of each prognostic predictive model is in progress.

Conclusion:

This systematic review examines the quality of published prognostic predictive models in MND. Provisional findings suggest most such models have methodological flaws, are not externally validated, have had limited adoption in clinical care and trials, and are not sufficiently transparent to allow studies to compare predictive performance between models. Based on our final findings, we will describe the current landscape of prognostic prediction models in MND and propose directions for future such projects.

180. Prognostic Value of Serum Troponin T Elevations in a real life Cohort of ALS Patients

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Introduction and Background: Serum levels of cardiac troponins T (cTnT) and I (cTnI) are highly specific biomarkers of acute myocardial injury. However, diseased skeletal muscle can be a source of cardiac Troponin T in chronic skeletal muscle disorders (1).

In ALS, cardiac TnT is elevated in > 60% of patients (2). It correlates with disease severity and was therefore suggested as a biomarker (2). Here we aimed to investigate whether cTnT levels have a prognostic value in ALS.

Methods:

In this retrospective cross-sectional study, we reviewed the real-world data from 108 patients from our ALS clinic at the University Hospital Bonn with a known date of death between 2020 and 2022.

Kaplan Meyer curves for patients with normal and elevated serum Trop T levels were generated and compared using the Log Rank test

Results:

We included 104 patients with a mean age of 68 years, out of which 40 % were female. Cardiac Troponin T was increased in 67 % of patients. The mean cTnT was 30,25 ng/ml (+/- 22,79), while cTnI was normal in >97% of the cases.

The log rank test revealed that there was a statistically different probability of survival between patients with normal and elevated serum troponin T. Patients with elevated serum troponin T had a hazard ratio of 3.629 (95% CI: 1.535-8.58).

Conclusion

Our study suggest that serum troponin T might be developed into a predictor of survival of ALS patients. We speculate that serum Troponin T might reflect some aspect of respiratory function, the main determinant of survival in ALS.

1: (du Fay de Lavallaz et al., 2022)
2: (Castro-Gomez et al., 2021)

181. Relationships Between Brain Iron and Blood Markers for Iron and Inflammatory Status in Cognitively Healthy Adults.

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Background:

Regional increases in brain iron have been observed in several neurodegenerative diseases, including Amyotrophic Lateral Sclerosis. Several studies show links between increases in brain iron and decline in cognitive function, however, the causes of brain iron increases remain unclear. This study evaluates the relationships between brain iron and blood markers for systemic iron and inflammatory status to gain a better understanding of brain iron regulation.

Methods:

Brain MRI scans and blood samples were collected from 328 cognitively healthy participants aged between 28 and 74 years old (152 male, 176 female). MRI scans were processed using quantitative susceptibility mapping to quantify regional brain iron. To assess systemic iron status, haematocrit, plasma ferritin and plasma soluble transferrin receptor (sTfR) were measured and total body iron index (TBI) was calculated. To assess systemic inflammatory status, C-reactive protein (CRP), Neutrophil : Lymphocyte ratio (NLR), plasma monoclonal colony stimulating factor 1 (MCSF), plasma interleukin 6 (IL6) and plasma interleukin 1β (IL1β) were assessed.

Results:

Males had significantly higher iron levels in the left and right hippocampus and left thalamus, while females had higher iron levels in the left and right caudate, right pallidum and left putamen. Females exhibited associations between TBI and iron levels in the left and right caudate and right pallidum. We also demonstrated associations between haematocrit and iron levels in the right pallidum and left putamen. However, no associations were observed between brain iron and blood iron levels in males. Males exhibited positive associations between IL6 levels and iron in the right amygdala and right pallidum, and a negative association between IL6 levels and iron in the left caudate. In males, a positive association was also observed between CRP levels and iron in the right thalamus. Positive associations between iron in the left thalamus and NLR were observed in both sexes.

Conclusions:

We only demonstrated links between systemic iron levels and brain iron in females but not males, whereas more links were observed between systemic inflammation and brain iron levels in males. These results suggest differing iron regulation mechanisms between sexes which could have implications on neurodegenerative disease mechanisms. Further research is necessary to determine the true nature of these relationships.

182. SerpinA1 as prognostic biomarker in Amyotrophic Lateral Sclerosis: an exploratory study

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Background and Objectives

SerpinA1, a serine protease inhibitor, is produced during the acute phases of inflammation and proved to be involved in the modulation of microglial-mediated inflammation in neurodegenerative diseases. Due to its potential protective role, we aimed to analyze SerpinA1 levels in CSF and serum of ALS patients to determine its prognostic value.

Methods

SerpinA1, Neurofilament Light (NfL) and Heavy (pNfH) Chain, chitinase-3 like-protein-1 (CHI3L1) were determined in CSF and serum of ALS patients (n=110) and healthy controls (n=10) by ELLA (semi-automated ELISA), and correlated with clinical parameters, after identifying three classes of progressors (fast, intermediate, slow) according to disease progression rate. Biomarkers levels were analyzed for diagnostic power and association with progression and survival.

Results

Contrarily to neurofilaments, SerpinA1 concentrations in serum and CSF did not correlate. Serum SerpinA1 was significantly decreased in ALS patients (median:1032 ug/ml, IQR: 658.9 - 1467.9) compared to controls (1343 ug/ml, IQR: 1008.1 - 3047.8, p=0.02). CSF SerpinA1 was elevated in fast progressors (8.6 ug/ml) compared to both slow (4.43 ug/ml) and intermediate (4.42 ug/ml) progressors (p-value 0.048). In multivariate analysis, the ratio between serum and CSF SerpinA1 (SerpinA1ratio), and CSF pNfH were independently associated with survival (HR: 5.16, 95%CI 2.34-11.36, p-value <0.001 for SerpinA1ratio), together with DPR and respiratory function (forced vital capacity, FVC). When stratifying according to cut-off values for CSF pNfH and SerpinA1ratio, they were confirmed as good predictors of survival: median survival was 26.7 months (95% CI:13.7–32.1) for CSF pNfH above 7354 pg/mL, and 37.4 months (95% CI:31.5–46.6) for values below (p=0.002); for SerpinA1ratio above 0.017 median survival was 20 months (95% CI:9-n.a.) and 35.3 months (95% CI:30.4-42.3) for values below (p=0.02).

Discussion

Our results support the notion that SerpinA1 may represent an independent and concurrent indicator of ALS prognosis, along with other biological markers of neurodegeneration as neurofilaments and clinical variables of established prognostic significance. These exploratory findings warrant further confirmatory studies in larger cohorts and collaborative longitudinal studies, including more controls, together with preclinical studies where SerpinA1 expression and role should be clarified in the setting of ALS.

183. T cell responses at diagnosis of amyotrophic lateral sclerosis predict disease progression

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, involving neuroinflammation and T cell infiltration in the central nervous system. However, the contribution of T cell responses to the pathology of the disease is not fully understood. Here we show, by flow cytometric analysis of blood and cerebrospinal fluid (CSF) samples of a cohort of 89 newly diagnosed ALS patients in Stockholm, Sweden, that T cell phenotypes at the time of diagnosis are good predictors of disease outcome. High frequency of CD4+FOXP3– effector T cells in blood and CSF is associated with poor survival, whereas high frequency of activated regulatory T (Treg) cells and high ratio between activated and resting Treg cells in blood are associated with better survival. Besides survival, phenotypic profiling of T cells could also predict disease progression rate. Single cell transcriptomics analysis of CSF samples shows clonally expanded CD4+ and CD8+ T cells in CSF, with characteristic gene expression patterns. In summary, T cell responses associate with and likely contribute to disease progression in ALS, supporting modulation of adaptive immunity as a viable therapeutic option.

184. Thoracic spinal cord gray matter atrophy as a sensitive marker for early ALS

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Background: In Amyotrophic Lateral Sclerosis (ALS), there is an urgent need for valid and reliable surrogate markers to facilitate the diagnostic process, to monitor disease progression, and to evaluate drug efficacy in upcoming trials. The novel radially acquired Averaged Magnetization Inversion Recovery Acquisitions (rAMIRA) method enables high-resolution images of both the cervical and thoracic spinal cord (SC) gray matter (GM), the latter of which is notoriously challenging to image due to an increased susceptibility for artifacts. The aims of this study were to evaluate the cervical and thoracic SCGM in patients with ALS at different disease stages and levels of diagnostic certainty.

Methods: Using rAMIRA imaging and a semi-automated segmentation approach (JIM7), we assessed cervical and thoracic SCGM areas at the intervertebral disc levels C2/3 – C5/C6 and at the lumbar enlargement (Tmax) of 36 ALS patients and 36 age- and sex-matched healthy controls (HC). We categorized patients' disease stages according to the King's staging method and to the level of diagnostic certainty using the revised El Escorial criteria.

Results: Both cervical and thoracic SCGM area at all levels were reduced compared to HC ($p < 0.001$), with relative reductions (rr) pronounced at the cervical (C3/C4, rr = 15.1%)) and lumbar enlargement (Tmax, rr = 20.2%). Categorizing patients using the King's staging method, we found patients at King's stage 3 to exhibit significant SCGM atrophy at the cervical and thoracic levels compared to HC, while patients at King's stage 1 showed significant SCGM atrophy only at Tmax. Stratifying patients according to the level of diagnostic certainty applying the rEE, patients with probable and definite ALS showed significant GM atrophy at all levels compared to HC, while patients with a possible ALS showed significant GM atrophy only at Tmax. Significant SCGM atrophy at Tmax could even be detected in the subgroup of patients with a bulbar onset of symptoms.

Conclusion: Cervical and thoracic SCGM evaluated via rAMIRA imaging show significant atrophy in a cohort of patients with ALS compared to HC. Patients in early disease stages show selective SCGM atrophy at Tmax, suggesting that the lumbar SCGM area may be especially to early changes and may aid in the diagnostic process and serve as an appealing surrogate in upcoming drug trials. Further evaluation of thoracic SCGM is needed to assess its value as a diagnostic marker.

185. Tongue strength as a biomarker of bulbar dysfunction and progression in ALS patients

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Introduction:

Bulbar symptoms appear at some point in the disease, with important implications for functional capacity, quality of life and the possibility of complications. In healthcare practice and clinical trials, its assessment is often performed through the functional scale (ALSFRS-R) and its items related to language, salivation, and swallowing. However, a single objective assessment that represents all three bulbar functions at the same time is lacking. Our aim is to assess whether the measurement of maximum tongue pressure (MTP) correlates with the bulbar dysfunction observed in ALS.

Methods:

Retrospective analysis of prospectively collected data from the registry of ALS patients visited between 2016 and 2022 in our center, in whom examination of MTP was performed, as well as functional data (ALSFRS-R) and Drooling Severity and Frequency Scale (DSFS).

Results:

288 MTP and ALSFRS-R assessments of 81 ALS patients were evaluated. 49 patients (60.5%) had spinal onset, the mean age at diagnosis was 64.4 years (SD: 12), and 45 (55.6%) were women. MTP adjusted correlated with the bulbar items separately; speech (R:0.708; $p<0.001$), salivation (R:0.560; $p<0.001$) and swallowing (R:0.675; $p<0.001$), as well as with the total bulbar ALSFRS-R score (R:0.740; $p<0.001$).

Interestingly, we found that MTP detected tongue weakness in patients with clinically spared language (37.8%), salivation (54.1%) and swallowing (39%) as assessed by the ALSFRS-R score.

Additionally, 317 MTP and DSFS assessments of the same 81 patients were evaluated, and we found an inverse correlation between MTP and the severity (R:-0.557; $p<0.001$) and the frequency (R:-0.561; $p<0.001$) of drooling based on DSFS items. All correlations were adjusted for age and sex.

Conclusions:

MTP is an objective and easy-to-perform measure that allows the detection of bulbar dysfunction in patients with ALS and is more sensitive than the ALSFRS-R bulbar items.

186. Transcranial Magnetic Stimulation (TMS) as a potential early biomarker of Amyotrophic Lateral Sclerosis (ALS): a 12-month longitudinal clinical study

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ALS is a neurodegenerative disorder that causes progressive muscular wasting, paralysis and death on average within three years of symptom onset. The discovery of new potential therapies is held back by the lack of biomarkers for timely diagnosis and reliable progression monitoring of the disease. Previous studies using TMS combined with single-channel electromyography (EMG) identified signatures of cortico-spinal hyperexcitability in ALS individuals and demonstrated that this subclinical biomarker of motor neurons dysfunction is an early pathogenic mechanism preceding irreversible muscular atrophy. However, how effectively different TMS parameters of abnormal cortico-spinal excitability track disease progression is less well understood. Our 12-month longitudinal study, consisting of five visits spaced three months apart, employs a novel electrophysiological approach called high-density TMS. This technique combines TMS with a 64-channel high-density surface EMG readout system, instead of the commonly employed single-channel EMG, to measure established TMS protocols probing cortical inhibition through the duration of the cortical silent period (CSP), the magnitude of short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). We have now measured CSP, SICI and ICF from the first dorsal interosseous (FDI) muscle of the dominant hand in 13 ALS patients (6 females, mean age 63.2±6.2) and 13 healthy controls (6 females, mean age 64.9±7.9) over a 6-month period. High-density EMG allows for superior coverage of muscular volume compared to single-channel EMG. This enabled us to re-characterize previously established TMS protocols into a 3D anatomical map of the FDI firing, even in cases of severely wasted muscles in ALS patients. Our preliminary findings suggest that ALS patients exhibit a reduction in SICI compared to healthy controls ($P<0.05$), indicating cortical hyperexcitability. We are currently investigating its potential utility as a marker of disease progression over a 12-month follow-up period. Once completed, this study has the potential to improve our understanding of the topographical distribution and temporal evolution of disinhibition or excess facilitation that is thought to underlie cortical-spinal hyperexcitability in ALS. Precisely determining how excitability abnormalities evolve and spread over time may help the progression of a detailed anatomical map of disease trajectory in ALS.

187. Treatment with Tofersen of a patient carrying a heterozygous novel frameshift SOD1 mutation (p.Ser108LeufsTer15) causing a premature termination codon

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Introduction: About 20% of familial Amyotrophic Lateral Sclerosis (ALS) cases are caused by mutations in the SOD1 gene [1], which can acquire both gain and loss of function. Gain of function may result in toxic SOD1 aggregates thus disrupting several cellular processes [1]. A minority of mutations cause premature termination codons (PTCs). mRNA harboring PTCs should be rapidly degraded by nonsense-mediated mRNA decay, which limits the production of truncated proteins, prone to induce misfolded protein or toxic aggregates but some of them escape from this process.

We present the case report of a 77-year-old female, affected by slow progressing ALS since 2010, carrying a heterozygous novel frameshift SOD1 mutation (p.Ser108LeufsTer15), causing a truncated protein [2]. It cannot be excluded that this mutation may trigger toxic mechanisms as a consequence of its propensity to aggregate [2]. We administered Tofersen to the patient, with a strictly clinical and biomarker assessment to monitor treatment's safety and efficacy.

Methods: Functional outcomes measures, such as ALSFRS-R, manual muscle testing and FVC, were collected at each intrathecal infusion of Tofersen. CSF and plasma levels of Neurofilament light chain (NF-L) were quantified using SiMoA technology at baseline and before each Tofersen administration.

Results: On November 2022 the patient started with the loading dose every 2 weeks and after 1 month she started the maintenance dose. No side effects or worsening related to the treatment were reported. CSF NF-L levels showed a 22% reduction from a median of 1089,26 pg/ml during the loading doses to a median of 846,75 pg/ml in the first three maintenance doses. Accordingly, plasma NF-L concentrations diminished of 25% from the loading phase (median NF-L=33,81 pg/ml) to the maintenance phase (median NF-L=25,17 pg/ml).

Conclusions: To the best of our knowledge this is the first case report of an ALS patient harboring a PTC mutation in SOD1 being treated with Tofersen. Our findings support the hypothesis that ALS patients carrying pathogenic mutant mRNA with PTCs may benefit from Tofersen therapy. Further evaluations of clinical and laboratory course of the patient in the next months will help to better clarify the benefit of Tofersen treatment in this kind of SOD1 mutation.

Bibliography: 1. doi: 10.1074/jbc.M209915200. Epub 2002 Dec 9.2003 2. doi: 10.1016/j.neurobiolaging.2018.08.014. Epub 2018 Aug 24. PMID: 30236613.v

188. Widespread alterations in Fast Amyotrophic Lateral Sclerosis Progressors: A Brain DTI and Sodium MRI Study

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BACKGROUND AND PURPOSE

While conventional MRI has limited value in amyotrophic lateral sclerosis (ALS), non-conventional MRI has shown alterations of microstructure using diffusion MRI and recently sodium homeostasis with sodium MRI. We aimed to investigate the topography of brain regions showing combined microstructural and sodium homeostasis alterations in ALS subgroups according to their disease progression rates.

MATERIALS AND METHODS

Twenty-nine patients with ALS and 24 age- and gender-matched healthy controls (HC) were recruited. Clinical assessments included disease duration and the revised ALS functional rating scale (ALSFRS-R). Patients were clinically differentiated into fast (n=13) and slow (n=16) progressors according to their ALFSRS-R progression rate.

3T MRI brain protocol included: (1) 1H T1-weighted and diffusion sequence; (2) 23Na density-adapted radial sequence. Quantitative maps of diffusion with fraction anisotropy (FA), mean diffusivity (MD) and total sodium concentration (TSC) were measured. The topography of diffusion and sodium abnormalities were assessed by voxel-wise analyses.

RESULTS

ALS patients showed significantly higher TSC and lower FA, alongside higher TSC and higher MD, compared to HC, primarily within the corticospinal tracts (CSTs), the corona radiata and the body and genu of the corpus callosum. Fast progressors showed wider spread abnormalities mainly in frontal areas. In slow progressors, only FA measures showed abnormalities when compared to HC, localized in focal regions of the CSTs, the body of corpus callosum, the corona radiata and the thalamic radiation.

DISCUSSION

This study highlighted brain regions with common microstructural and sodium homeostasis disturbances corresponding to relevant regions involved in ALS. Interestingly, fast progressors showed widespread combined alterations while slow progressors only showed restricted microstructure damage.

Recently, increasing evidences emphasized that heterogeneous disease progression rates influence diagnosis, prognosis and might affect the responsiveness to treatment. Therefore, there is an urgent need of patient stratification. Our results confirm previous reports showing that non-conventional and multiparametric MRI technics might contribute to the diagnostic work-up of patients with different clinical profiles, especially when combined with mathematical modeling and artificial intelligence to predict disease progression trajectory of a single patient.

Clinical research

189. A Case of Juvenile Amyotrophic Lateral Sclerosis Carrying variants in TARDBP and NEK1: A peculiar phenotype and discussion of oligogenic model in ALS

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Amyotrophic lateral sclerosis (ALS) can rarely affect young individuals. Juvenile ALS (JALS) is defined as individuals with an onset of the disease before the age of 25. One of the differences between adult-onset ALS and JALS is their genetic background. A higher contribution of genetic causes is detected in JALS than in adult-onset ALS, and the proportions of associated genes are also different. Additionally, a multi-hit mechanism has been postulated as a pathophysiological process in ALS in which an oligogenic model may play a significant role. We present a case report of JALS with a pathogenic variant in the TARDBP gene, previously described only in adult-onset ALS and with an atypical phenotype of marked upper motor neuron predominance. The patient also carried a missense variant of uncertain significance (VUS) in the NEK1 gene. In addition, we performed a literature review of JALS cases associated with variants in the TARDBP gene to discuss the phenotype and possible implications of the variant in NEK1.

A 24-year-old male patient presented with a progressive disorder of rigidity and weakness of the lower limbs. The electrophysiological tests showed evidence of lower motor neuron involvement and a conduction defect in the corticospinal pathway in both upper and lower extremities. The patient developed a rapid progression with a pyramidal syndrome that spread to the upper extremities and bulbar region leading to spastic tetraparesis, dysarthria and dysphagia. The patient required percutaneous endoscopic gastrostomy placement 15 months after diagnosis. There was no family history of neurological or psychiatric disorders. The patient was an only child born of non-consanguineous and clinically unaffected parents. Genetic studies detected an heterozygous variant (p.Asn345Lys) in the TARDBP gene, previously described in only 4 patients with adult-onset ALS. An additional heterozygous variant (p.Phe987Leu) in NEK1 was identified and classified as a VUS. Segregation studies showed a paternal origin of the TARDBP variant, while the NEK1 variant was inherited from the mother.

This report highlights the heterogeneity of the phenotypic presentation of ALS associated with diverse pathogenic genetic variants. Given the peculiarities of the phenotype, penetrance, and age of onset, we hypothesize that the NEK1 variant acts as a disease modifier in our case. This statement would require functional studies exploring an interaction between variants in both genes.

190. A multicenter prospective observational study to develop low-burden high-frequency prognostic digital speech biomarkers in ALS and FTD – PROSA

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There is a need for novel biomarkers indicative of disease state, projected progression, or response to treatment for amyotrophic lateral sclerosis (ALS) and associated neurodegenerative diseases such as frontotemporal dementia (FTD). Digital biomarkers are especially promising as they can be collected non-invasively and at low burden for patients. Speech biomarkers have the potential to objectively measure cognitive, motoric as well as respiratory symptoms at low cost and in a completely remote fashion using standard, widely available technology such as telephone calls, making them particularly appropriate for exceptional situations like the global COVID-19 pandemic.

The PROSA study aims to develop and evaluate low-burden high-frequency prognostic digital speech biomarkers. The main goal is to create a single, easy-to-perform battery that serves as the best proxy possible for cognitive, respiratory, and motor domains in ALS and FTD. To accelerate this development, PROSA will be a multicenter 12-months observational study aiming to include 75 ALS and 75 FTD patients as well as 50 healthy controls. The PROSA study builds on top of two established longitudinal cohorts of the German Research Center for Neurodegenerative diseases DZNE: DESCRIBE-ALS and DESCRIBE-FTD. Adding on to the extensive clinical phenotyping of DESCRIBE, PROSA collects a comprehensive speech protocol in fully remote and automated fashion over the telephone at four time points during one year. In combination with the extensive gold standard clinical phenotyping, the collected longitudinal speech data will allow advanced speech analysis using artificial intelligence (AI) for the development of speech-based phenotypes of ALS and FTD patients measuring cognitive, motor and respiratory symptoms. Such speech-based phenotypes can then be used to develop prognostic models predicting clinical change within the 1-year follow up using state-of-the-art machine learning models.

Results are expected to have implications for future clinical trial stratification as well as supporting innovative trial designs in ALS and FTD.

191. A MULTI-CENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED MULTIPLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF QRL-201 in ALS

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Introduction

A hallmark of amyotrophic lateral sclerosis (ALS) in >90% of patients, is the presence of cytoplasmic TAR DNA Binding Protein 43 (TDP-43) aggregates and loss of nuclear expression. Loss of nuclear TDP-43 leads to Stathmin-2 (STMN2) mis-splicing, resulting in decreased expression in the majority of people living with ALS. STMN2 is important for neural repair, axonal stability, and neuromuscular junction innervation. Our therapeutic approach is to correct STMN2 mis-splicing, thereby restoring protein function. QurAlis has shown that this can be accomplished using a splice switching antisense oligonucleotide (ASO) in non-clinical studies. QRL-201 is an investigational ASO that rescues STMN2 expression and protein function in QurAlis' patient-derived neuronal disease models, even in the continued presence of TDP-43 pathology.

Methods

QurAlis has designed a phase 1 study evaluating the safety and tolerability of multiple doses of QRL-201 in people living with ALS. QRL-201-01 is a double-blind, multiple-ascending dose study in which approximately 64 people living with ALS will receive QRL-201, or matching placebo, in a 6:2 ratio. The study design includes six dose escalation cohorts and two exploratory cohorts. This study will include the collection of data on numerous biomarkers, will employ sentinel participant dosing, and include multiple safety reviews.

Results

The primary endpoint will be incidence of adverse events. The secondary endpoints will be measurements of multiple dose pharmacokinetics (PK). This study will include multiple exploratory endpoints – 1) biomarkers of neuronal loss and STMN2 biology; 2) clinical outcome measures (ALSFRS-R, ROADS, SVC, HHD, electrophysiology testing); and 3) CSF PK profile.

Conclusion

QurAlis' mission is to bring breakthrough precision medicine technology to people living with ALS. QRL-201-01 is designed to evaluate the safety and tolerability of multiple doses of QRL-201 in people living with ALS, and explore the hypothesis that restoration of STMN2 is a suitable disease-modifying approach in ALS.

192. A MULTI-OMICS APPROACH TO STUDY MONOZYGOTIC TWINS DISCORDANT FOR AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterised by progressive death of upper and lower motor neurons, whose aetiology is still partially understood. The majority of ALS cases are sporadic (sALS), while 10% are familial (fALS). To investigate genetic and epigenetic factors underlying ALS, we studied a monozygotic twin pair discordant for ALS with a multi-omics approach, combining whole exome sequencing with genome-wide methylome- and transcriptome data from whole blood and PBMCs. For methylation, we used the Illumina EPICArray and ChaAMP software for the analyses, while for gene expression study Illumina TruSeq Stranded mRNA sequencing was performed. Results of the three omics were considered independently and in combination. We identified 59 differentially expressed genes (DEGs) (p.adj < 0.1; |log2FC| > 1) and confirmed the up or downregulation for 6 of them by ddPCR. Functional analyses on DEGs by different tools revealed the involvement of adaptive and innate immune system pathways. After QC, we found 2 differentially methylated probes (DMPs) (p.adj ≤ 0.1) in CACNA1G and VAX1 genes; while filtering by delta beta (Δβ) values, we identified 2 probes with Δβ ≤ - 0.25 (in an intergenic region and RUSC1-AS1) and 2 probes with Δβ ≥ 0.25 (in AARS and KPNA4). None of them fell into the highlighted DEGs. We validated DMPs identified by ChAMP by methylation-specific droplet digital PCR combined with methylation-dependent restriction enzyme (ddMSP). In particular, for the probe in VAX1 gene we confirmed the hypomethylation of the ALS twin. These results were compared with different larger literature datasets that included sALS and fALS patients, non-symptomatic FUS and C9ORF72 mutation carriers and healthy controls, without finding any correspondence at DMP and pathway levels. For exome analyses, ExomeDepth and ClassifyCNV identified 3 deletions and 1 duplication of uncertain significance in the ALS twin. Analyses of SNV, after filtering for frequency (≤ 0,00005) and QC (PASS), identified 25 variants classified as VUS (n=18) or likely benign (n=7). In conclusion, we integrated different omics performing functional analyses with several bioinformatic tools that underlined a possible role of the immune system in the disease. Further understanding of these immunological results and the validation of DMP in CACNA1G are ongoing to elucidate possible somatic genetic factors that could underlie susceptibility to sporadic ALS.

193. A novel GRN mutation in an Italian patient with non-fluent variant of primary progressive aphasia at onset: a longitudinal case report

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Objectives. We report the clinical presentation and evolution of a case with a novel Progranulin gene (GRN) mutation and non-fluent language disturbances at onset.

Methods. A 60 years-old Caucasian woman was followed due to a history of language disturbances. Eighteen months (M18) after onset, she underwent FDG positron emission tomography (PET), and at M24 she was hospitalized to perform neuropsychological evaluation, brain 3T MRI, lumbar puncture for cerebrospinal fluid (CSF) analysis, and genotyping. At M31, she repeated the neuropsychological evaluation and brain MRI.

Results. At onset the patient complained prominent language production difficulties, such as effortful speech and anomias. At M18, FDG-PET showed left fronto-temporal and striatal hypometabolism. At M24, the neuropsychological evaluation reported prevalent speech and comprehension deficits. Brain MRI reported left fronto-opercular and striatal atrophy, and left frontal periventricular white matter hyperintensities (WMHs). Increased CSF total tau level was observed. Genotyping revealed a new GRN p.H340TfsX21 mutation. She received a diagnosis of nonfluent variant of primary progressive aphasia (nfvPPA). At M31, language deficits worsened, together with attention and executive functions. She presented also with behavioural disturbances, and a progressive atrophy in the left frontal-opercular and temporo-mesial region.

Discussion. The new GRN p.H340TfsX21 mutation resulted in a case of nfvPPA characterized by fronto-temporal and striatal alterations, typical frontal asymmetric WMHs, and a fast progression towards a widespread cognitive and behavioral impairment, which reflects a frontotemporal lobar degeneration. Our findings extend the current knowledge of the phenotypic heterogeneity among GRN mutation carriers.

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194. A Novel Machine-Learning Based Clinical Trial Subgroup Analysis Tool

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Introduction

We previously developed machine-learning based ALS predictive models1-4, including a time to 50% expected VC (VC50) model. Here we report on the use of the VC50 model to develop a novel subgroup analysis tool that we call "Detectable Effect Cluster" (DEC) analysis.

Methods

The VC50 model was used to rank-order a randomly selected group of 300 PRO-ACT patients (the "trial" population) by predicted log-likelihood. The trial was split 1:1 into "treatment" and "placebo" arms and a simulated 20% slowing in rate of ALSFRS-R progression correlated to creatinine level was applied to the treatment arm. Risk groups using predicted log-likelihood thresholds were defined by systematically expanding subgroups in 2% increments until all participants were included, thus building a series of 1,250 subgroups. To analyze all subgroups, a matrix was plotted in which each block was derived using distinct upper and lower percentile limits. For each subgroup created in this way we performed a statistical analysis, thus developing a "heat map" to reveal combinations of patient subgroups within which statistically significant effect sizes ($p<0.05$) were detectable.

Results

DEC heatmap matrices displaying the results from a simulated clinical trial were plotted for mean square error (MSE), treatment effect (TE), effect size and p-value. The plots revealed differentially stratified patient subgroups characterized by reduced MSE or elevated TE and effect size. The p-value heat map clearly defined a zone, a "hot spot," comprised of patients in the medium-to low risk group strata with a p-value less than 0.05.

Conclusions

Detectable Effect Cluster (DEC) analysis shows great promise in identifying subgroups within failed trials that could have formed the basis for successful trials. Importantly this approach allows investigators to explore the possibility of detecting subgroups in which a significant effect size is detectable both via reduced MSE or increased TE. One can also envision an adaptive trial with broad inclusion criteria, the purpose of which is to apply DEC analysis to identify a subgroup with a demonstrable treatment effect.

Acknowledgements

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195. A Phase 2 Trial of Pridopidine in ALS

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Background: Pridopidine is a highly selective sigma1 receptor agonist active in multiple models of human neurodegenerative disease. Phase 3 testing for HD is ongoing. We report evaluation of pridopidine in the HEALEY ALS Platform Trial.

Methods. 808 participants screened for 4 regimens of the platform trial; 179 assigned to the prodipine regimen. Participants had El Escorial possible, probable, or definite ALS, symptoms <36 mo from baseline, and VC >50%-predicted. Of 163 participants randomized, 121 received pridopidine and 42 placebo. An additional 122 placebo participants from 3 other concurrent regimens completed the Full Analysis Set (FAS) placebo arm. Double-blind treatment duration was 24 weeks. The primary endpoint was change from baseline through 24 weeks in ALSFRS-R total score using a Bayesian shared-parameter model of ALSFRS-R and mortality. There were multiple other prespecified and post-hoc endpoints. A subgroup of those with definite ALS and <18 mo symptom was prespecified; a number of post-hoc subgroups were also queried. Nominal p-values uncorrected for multiple comparisons are reported.

Results: Pridopidine was well tolerated. There were no significant differences between pridopidine and placebo participants in primary or key secondary outcomes. Pridopidine treatment showed improvements in speaking rate (p=0.028), articulation rate (p=0.013) , phonation time (p=0.076), and articulatory precision (p=0.11). Progression was slower among pridopidine vs. placebo particpants with definite ALS and <18mo duration by several measures including ALSFRS-R total score (p=0.19), bulbar domain (p=0.23), and respiratory domain (p=0.18). Similar patterns were seen for this subgroup in ALSAQ-40 total (p=0.018), the eating/drinking domain (p=0.015), and the communication domain (p=0.12). NfL declined modestly in all pridopidine-treated participants compared to placebo; a larger difference was noted in participants identified post-hoc with early onset and fast prestudy progression rate. In the same group, reduction in NfL was associated with a substantial effect on ALSFRS-R at 24 weeks.

Discussion: This study did not meet its primary endpoint. However, signals were consistently seen across outcomes related to speech in the FAS and in ALSFRS-R total, respiratory, bulbar and quality of life in a subset of patients with baseline characteristics similar to a recent pivotal ALS study. These data warrant further evaluation of pridopidine in a phase 3 study.

196. A randomized, double-blind, placebo-controlled, phase 2 study to assess safety, tolerability, and efficacy of RT001 in patients with ALS

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Objective: To evaluate the safety, tolerability, and initial efficacy of RT001, a deuterated homologue of linoleic acid, in patients with amyotrophic lateral sclerosis (ALS).

Methods: We conducted a randomized, multicenter, placebo-controlled clinical trial. Patients with definite, probable, or probable laboratory-supported ALS were randomly allocated to receive either RT001 or placebo twice a day for 24 weeks. After the double-blind period, all patients received RT001 during an open-label phase for 24 weeks. The primary outcome measures were safety and tolerability. Key efficacy outcomes included the ALS functional rating scale (ALSFRS-R), %predicted slow vital capacity, and plasma neurofilament light chain concentration.

Results: In total, 43 patients (RT001=21; placebo=22) were randomized. RT001 was well tolerated; one patient required dose reduction due to adverse events (AEs). Numerically, there were more adverse events in the RT001 group compared to the placebo group (71% versus 59%, p = 0.53), with gastrointestinal symptoms being the most common (43% in RT001, 27% in placebo, p = 0.35). Two patients in the RT001 group experienced a serious AE, though unrelated to treatment. The least-squares mean difference in ALSFRS-R total score at week 24 of treatment was 1.90 (95% CI: -1.39 to 5.19) in favor of RT001, p = 0.25. The directions of other efficacy outcomes favored RT001 compared to placebo, although no inferential statistics were performed.

Conclusions: Initial data indicates that RT001 is safe and well-tolerated among patients with ALS. Given the exploratory nature of the study, a larger clinical trial is required to evaluate its efficacy.

197. A weight loss risk prediction model in ALS

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Introduction

Amyotrophic lateral sclerosis (ALS) is typically associated with a degree of weight loss, potentially attributable to dietary changes due to dysphagia, muscle loss and hypermetabolism, though there are emerging premorbid factors. Weight loss is associated with shortened survival in ALS and is a focus of multidisciplinary care. The ability to accurately predict risk of weight loss in ALS would allow targeted nutritional support.

Methods

Longitudinal weights from 2,728 patients were obtained from the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database, and randomly partitioned into train and test subsets. Data from each first visit provided input variable data, and subsequent visits provided longitudinal weight information for time-to-event and censoring analysis. The preselected outcome variable of interest was 10% weight loss from baseline. Cox proportional hazard, accelerated failure time, and random survival forest models were built using the train subset baseline variables of age, sex, site of onset, time since onset, diagnostic latency, body mass index, forced vital capacity (as a percentage of predicted), and ALS Functional Rating Scale-Revised (ALSFRS-R) subscores.

Results

The accelerated failure time model proved most accurate in predicting the risk of 10% weight loss in the test subset, with an inverse probability censoring weighting (IPCW) Brier score = 0.148 (versus Cox proportional hazard and random survival forest scores = 0.150, and Kaplan Meier estimator = 0.164). Significant baseline predictors of 10% weight loss in the accelerated failure time model were reduced forced vital capacity (coefficient of one standard deviation change = -0.268, $p < 0.001$), reduced ALSFRS-R respiratory (coef = -0.164, $p < 0.01$) and leg (coef = -0.147, $p < 0.01$) subscores, increased time since onset (coef = 0.123, $p < 0.05$) and increased age (coef = 0.082, $p < 0.05$).

Discussion

Predicting a more individualised weight loss trajectory in ALS based on single-timepoint clinical variables is inaccurate. The accelerated failure time model will be further appraised using external data.

198. Acceptability of a Wearable Device to Evaluate Progression in People with Motor Neuron Disease

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Background: Motor neuron disease (MND) progression is traditionally evaluated using the revised ALS Functional Rating Scale (ALSFRS(R)), a questionnaire-based assessment.. There is an urgent need for more objective, detailed and sensitive measures of physical function to monitor clinical and research outcomes. Wearable devices that can quantify physical activity and motor change offer an opportunity to address this need. Accelerometers, such as the ActiGraph, are types of wearable device that evaluate movement of the limbs, with potential utility in monitoring change in motor function in degenerative disorders.

Aim: To investigate participants with MND's expectations and experiences of wearing a device during motor assessments, daily activity and whilst asleep.

Method: People with MND completed 12 weeks of fortnightly study visits whilst wearing an ActiGraph accelerometer on their right wrist and right ankle. Visits involved movement tasks, the 6 minute walking test and a 24 hour period of continual wear to explore general activity and sleep. Participants completed 3 questionnaires on use of health technology, expectations for devices and feedback on their experience of the accelerometers.

Results: 10 people with MND participated. All participants completed the full protocol, with seven data-points of motor assessments and 24-hour wear time periods each over the 12 week study. 70% reported having used wearable devices previously. All participants were excited about the prospect of trying a new technology. 80% of participants found wearing the devices to be a positive experience. No participants reported side effects, interference with daily living or added burden by study participation. Only one participant reported wearing these devices interfered with their sleep. One participant considered the devices inconvenient or uncomfortable, and one individual reported difficulties putting on their devices and increased the burden on their caregiver. By week 12, 30% of participants had experienced technical issues.

Conclusions: All participants were supportive of the integration of technology to monitor health. The findings of this study suggest that whilst people with MND can benefit from wearable devices, the suitability of the ActiGraph specifically must be explored further. Participants were a highly motivated, physically well and technologically literate subset of individuals, hence additional exploration of acceptability outside this group is n

199. Acces barriers for dignified death through euthanasia in Colombia

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The fundamental right to die with dignity represents the faculties that allow a person to live the end of their life cycle with dignity. Euthanasia is legal and a right in Colombia but people with ALS face multiple barriers. even when the criteria of a verbal or written, informed, voluntary, unambiguous, and persistent request are met.

We describe 14 patients who requested euthanasia between 2020 and 2022, seven of them are women, with an average age of 66 years, ALSFRSR average 17. Ten patients died in the euthanasia procedure.

Access barriers are described as the lack of education in medical professionals, authorization procedures with insurers, geographical barriers, and religious characteristics.

200. Adolescents' need for professional support when living with a parent with ALS – based on both the adolescents' and the parents' experiences

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Background: Living with a family member who is progressively deteriorating due to a life-threatening condition such as amyotrophic lateral sclerosis (ALS) can be burdensome and demanding. Witnessing how a parent is affected by the disease might be extra difficult for adolescents, partly because they are already in a particularly vulnerable period of life. Although ALS probably has an impact on adolescents as relatives, research on how they should be supported is limited. More knowledge is therefore required about their specific need for professional support to promote or maintain their health.

Subject: The aim of the study was to illuminate adolescents' need for professional support when living with a parent who has ALS – based on both the adolescents' and the parents' experiences.

Methods: The study draws on 37 individual in-depth interviews with 18 families from different cities in Sweden, including 11 adolescents and 26 parents, 13 with ALS and 13 co-parents. The adolescents were between the ages of 8 and 25, and fully or partially lived together with a parent with ALS. The interview data were analysed with qualitative content analysis.

Results: The results show a need for professional support among the adolescents to bring some manageability into their life situation marked by great uncertainty. Their need for support seemed to be both individual and varied depending on the progression and phase of the disease, but also difficult to articulate. Getting help from healthcare in making the needs visible was considered urgent, in order to be offered individually adapted support. The adolescents' need of obtaining information and understanding was addressed, and accordingly, the importance of involving them in discussions about their parents' condition, the development of the disease and possible future scenarios. A more active approach from healthcare was also requested in both reaching out to the adolescents as early as possible, but also in gathering the whole family to facilitate communication and to strengthen them in dealing together with challenges that life with ALS might entail.

Conclusions: The adolescents' individual and disease-dependent need for professional support reinforce the importance of meeting them at their own level and based on each family's unique situation. Clearer guidelines are needed for professionals who meet these families to ensure that the adolescents and their needs are not overshadowed in the context of the d

201. ADULT-ONSET OF FUS-RELATED AMYOTROPHIC LATERAL SCLEROSIS PATIENTS: PHENOTYPE AND GENOTYPE CHARACTERIZATION

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FUS (Fused in sarcoma) is a gene involved in various callular processes, including DNA and RNA processing, transcriptional regulation and transport of RNA molecules. Mutations in the FUS gene have been linked to amyotrophic lateral sclerosis (ALS). Eventhough FUS mutations are mainly related to familial early onset ALS forms with an aggressive course, some cases of both sporadic and familiar adult onset have been descibed.

We identified and analysed the clinical characteristics of 14 FUS mutated ALS patients identified from a single-center cohort of 1182 genetically tested ALS patients: 3 patiens presented a juvenile ALS onset, while 11 patients showed an adult onset disease.

Among the eleven patients with an adult onset of ALS the mean mean age of onset was 51.24 years [43.66-68.05]. 3 unrelated patients (27.27%) had a reported familiy hisotry of ALS. The onset was on bulbar site on 5 patients (45.45%), spinal site on 6 (54.55%). Median tracheostomy-free survival time was 57.50 months (8.27-90.37). Nine known mutations have been found: p.R521C was the most frequent (3 patients out of 11); the other mutations found were p.R522G(1pt), p.M524V(1pt), p.R491C(1pt), p.P525L(1pt), p.G230C(1pt), p.G509D(1pt), p.G245V(1pt), p.D490N(1pt). Interestingly, in 3 patients a second mutation, respectively one in TDP43 and two with pathological expansions of C9orf72 gene, has been detected.

Considering these data we can observe that FUS mutations should be considered also in adult onset ALS patient even with no family history of ALS. As expected, our cohort showed a high genotipic heterogeneity with prevalence of p.R521C mutation. Interestingly, the possible adding effect due to other mutations in the ALS related genes, has been documented. Clinical characteristics confirm a less agressive behaviour of adult onset compared to the juvenile form. Overall, our datase confirm the relevance of a comprehensive clinical and genetic evaluation in all ALS patients to better characterized patients population and select the right cohort for future treatments.

202. Age and gender distribution in three aggressiveness strata in 653 Thuringian ALS patients

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Background: Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive neurodegenerative disorder with a similar gender ratio and a median age of disease diagnosis of 55. However, data about the age and gender composition in high, intermediate and low aggressive ALS is scarce.

Objectives: Visualization of different disease courses and aggressivities in gender ratio and age of disease onset based on the D50 model.

Methods: Between August 2007 and May 2021 patients with ALS determined by the revised El Escorial criteria were recruited at the Department of Neurology, University Hospital Jena, Germany. After obtaining informed written consent, we collected 4066 collected ALSFRS-R in 653 individuals to perform iterative fitting in the D50 model as previously described. The group was divided into two age groups relating to the international age of disease diagnosis median of 55 years. Based on D50 the disease aggressivity was divided into High (D50 <20), Intermediate (20 ≤ D50< 40) and Low (D50 ≥ 40). SPSS (Ver. 28.0; IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. The visualization of the data analysis was accomplished by Microsoft Excel.

Results: The analyzed cohort consisted of 291 females and 362 males with a median age of disease onset of 63.1 in females and 61.3 in males. A frequency scale of aggressivity was provided as following: low 24.2%, intermediate 43.6%, high 32.2% with a similar gender ratio. The group of patients under 55 years by the time of diagnosis included 37.4% females and 62.6% males. The group of patients from 55 years contained 46.7% females and 53.3% males. In the elderly group the percentage of high aggressivity was higher than in the younger group (24.5% <55 y, 34.6 >=55 y p<0.006).

Conclusion: Assuming that the D50 model maps the aggressivity of the ALS we could show that in our cohort the difference between the male and female patients referring to the disease course was not statistically significant. However, eldery patients tend to suffer from high aggressive disease courses more frequently, the younger group provided a higher percentage of male subjects. The collection of the ALSFRS-R took place in the outpatient regular clinical care so the number of ALSFRS-R scores varied between subjects.

203. ALS and the Gut-Brain Axis: A systematic review and meta-analysis assessing the relationship between amyotrophic lateral sclerosis, the gut and its mi

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Amyotrophic lateral sclerosis is a neurodegenerative disease with central nervous system (CNS) manifestations affecting motor and cognitive function. However, recent research has highlighted that non-CNS manifestations can also occur with evidence of protein misfolding pathology affecting the gastrointestinal tract. Indeed, the influence of the gut-brain axis in neurodegenerative conditions, including Alzheimer's disease and Parkinson's disease, as well as ALS, is becoming increasingly pertinent as these non-CNS manifestations may precede CNS symptoms in people with these conditions. Here we present a systematic review and meta-analysis assessing the role of the gut-brain axis in ALSFTSD including preclinical and clinical studies examining both the microbiome and non-microbial pathological pathways. Database searches of Medline, PubMed, and Embase were performed using pre-determined search terms. Twenty-five studies were included in the final quantitative meta-analysis. Studies examining differences in the microbiome between (i) people with ALS and controls and (ii) preclinical models of ALS and controls, show no significant differences, largely due to small sample sizes and inter- and intra-study inconsistencies. However, interventions targeting gut microenvironment and/or gut-resident pathologies demonstrate a significant improvement in disease outcomes, such as motor function and survival. Our data suggest that interventions either targeting the gut microenvironment or alleviating gut pathology are a promising new therapeutic avenue for future research.

204. ALSFRS-R-SE: an adapted, annotated, and self-explanatory version of the revised amyotrophic lateral sclerosis functional rating scale

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Background:

The ALS Functional Rating Scale in its revised version (ALSFRS-R) is a disease-specific severity score that reflects motor impairment and functional deterioration in people with amyotrophic lateral sclerosis (ALS). It has been widely applied in both clinical practice and ALS research. However, in Germany, several variants of the scale, each differing slightly from the others, have developed over time and are currently in circulation. This lack of uniformity potentially hampers data interpretation and may decrease item validity. Furthermore, shortcomings within the standard ALSFRS-R questions and answer options can limit the quality and conclusiveness of collected data.

Methods:

In a multistage consensus-building process, 18 clinical ALS experts from the German ALS/MND network analyzed the ALSFRS-R in its current form and created an adapted, annotated, and revised scale that closely adheres to the well-established standardized English version.

Results:

Ten German-language variants of the ALSFRS-R were collected, three of which contained instructions for self-assessment. All of these variants were compiled and a comprehensive linguistic revision was undertaken. A short introduction was added to the resulting scale, comprising general instructions for use and explanations for each of the five reply options per item. This adapted version of the scale, named ALSFRS-R-SE (with the “SE” referring to “self-explanatory”), was carefully reviewed for language and comprehensibility, in both German and English.

Conclusion:

An adapted and annotated version of the ALSFRS-R scale was developed through a multistage consensus process. The decision to include brief explanations of specific scale items and reply options was intended to facilitate ALS-FRS-R-SE assessments by both healthcare professionals and patients. Further studies are required to investigate the accuracy and utility of the ALSFRS-R-SE in controlled trials and clinical real-world settings.

205. Alterations of spontaneous speech in primary progressive aphasia variants: a neuropsychological and brain MRI study

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Objectives. The aims of the study were: 1)to identify which features of spontaneous speech most effectively distinguish PPA variants (non-fluent/agrammatical [nfvPPA], semantic [svPPA], and logopenic [lvPPA]) from healthy controls (HC) and each other; 2)to determine whether the speech measures associated to each variant are related to gray matter (GM) density of specific language brain circuits.

Materials. 95 patients with a diagnosis of PPA (40 nfvPPA, 35 svPPA, and 20 lvPPA) and 25 HC underwent a neuropsychological assessment, the audio-recorded 'Picnic Scene' test from the Western Aphasia Battery, and a brain MRI.

Stepwise regression models detected the best speech parameters able to distinguish the groups. Multiple regressions were performed between GM volumes and global z-scores of each 'best model' resulting from the Stepwise Regression analysis.

Results. The best model differentiating PPA patients and HC included: false starts, mean production rate, mean frequency, and length of sentences for lvPPA (R2=0.731); production rate and self-corrected sequences for nfvPPA (R2=0.665); mean frequency of produced nouns for svPPA (R2=0.466). In lvPPA, nfvPPA and svPPA, z-scores of each 'best-model' variables were positively associated with the GM density of left postcentral, inferior frontal, and inferior temporal gyri, respectively. The best model to distinguish: lvPPA from nfvPPA cases included incomplete and subordinate sentences (R2=0.295); lvPPA from svPPA included naming and repetition, proportion of sentences and verbs (R2=0.646); nfvPPA from svPPA included naming, repetition, number of produced verbs and sentences, and production rate (R2=0.757).

Conclusions. In the comparisons among PPA variants, the best models were reached when distinguishing svPPA cases from each other variant, mainly when also standard language tests were included in the model. The accuracy in distinguishing lvPPA and nfvPPA cases is still low; however, the proposed models could benefit from including other biomarkers, such as the brain MRI measures. The speech variables that we identified and that were related to specific GM circuits, may be used in the clinical practice for patients' differential diagnosis, prognosis, and planning pharmacological and non-pharmacological interventions.

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206. Amyotrophic Lateral Sclerosis and Lead

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Objective: In this study we investigate of lead an Amyotrophic Lateral Sclerosis (ALS).
Material-

Method: In this study, 131 patients were examined in 2 groups (A-76 regular group B-55 person irregular group) after being followed up in Istanbul Medical Faculty Neurology Department between 1970-1990 and diagnosed with ALS according to El Escorial criteria. The male/female ratio was 1.62. The age range of disease onset ranged from 15 to 73. The mean age at onset was 48.7. Most of the patients came from the Marmara region. Blood and urine lead levels were measured in all 131 patients, and each family member of these patients was taken as a control group and compared. 16 of them were in the PBP, 72 in the bulbar ALS, and 43 in the spinal ALS group. The results and findings in 131 patients with classical ALS were consistent with the literature, except that the mean age of onset was early. A very small dose (diagnostic dose = 0.2 – 0.4 mg TRH) was administered to 26 regularly controlled patients as a TRH treatment trial. It was administered intravenously for 6 months, as 1 ampoule every 7-10 days.

Conclusion: We showed that lead is a risk factor of ALS and we have seen the treatment trials were not effective of the diseas and diseas duration.

Keywords: Amyotrophic Lateral Sclerosis, Lead,Treatment, Turkiye, TRH

207. An observational cohort study on pulmonary function in adult patients with 5q-spinal muscular atrophy under nusinersen therapy

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Background: Few studies assessed the effect of nusinersen on respiratory function in adult patients with spinal muscular atrophy (SMA). The aim of this single-center study was to analyze pulmonary function and its association with muscle function and quality of life (QoL) in adult patients with 5q-SMA under nusinersen.

Methods: We recorded forced vital capacity (FVC), forced expiratory volume in the first second (FEV1) and peak expiratory flow (PEF) during nusinersen treatment in 38 adult SMA patients. Revised Upper Limb Module (RULM), Hammersmith Functional Motor Scale Expanded (HFMSE), 36-Item Short Form Health Survey (SF-36) questionnaire and Fatigue Severity Scale (FSS) were recorded and correlations between muscle function, QoL, fatigue and respiratory parameters were analyzed.

Results: No differences were detected between mean FVC, FEV1, PEF at different timepoints versus baseline. Ambulatory patients showed significant improvement in mean PEF at month 30, compared to non-ambulatory patients (+0.8 ± 0.5 vs. -0.0 ± 0.5, $p<0.05$). Patients without fatigue at baseline showed significant improvement in mean PEF at month 10, compared to patients with fatigue at baseline (+0.4 ± 0.5 vs. -0.6 ± 0.9, $p<0.05$). Physical domains of SF-36 positively correlated with the change in FVC and FEV1. FSS negatively correlated with the change in mean PEF.

Conclusion: Mean pulmonary function remained stable during nusinersen treatment over a period of up to 30 months. Non-ambulant patients and patients with fatigue at baseline should be monitored for pulmonary function during nusinersen treatment. Improvement in pulmonary function was associated with improvement in motor function, fatigue and QoL, early after nusinersen initiation.

208. Analytical Validation of Siemens Healthineers Atellica® IM NfL Assay and its Clinical Utility in People with SOD1-ALS in the VALOR Study (PREF POSTER)

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Neurofilaments (NFs) are major components of the neuronal cytoskeleton that are released into the extracellular space following axonal degeneration resulting in elevated levels in both cerebrospinal fluid (CSF) and blood. In ALS, NF elevations exceed nearly all other neurodegenerative diseases. Higher levels of NFs correlate with faster disease progression and shortened survival. Here we present the analytical performance of a NF light chain (NfL) assay on the Siemens Healthineers Atellica® IM and its clinical utility in detecting CSF and plasma NfL in the Biogen sponsored ALS VALOR study (NCT02603699).

The NfL assay was analytically validated for serum, plasma, and CSF on the Atellica® Solution. The assay ranges for EDTA plasma and CSF are 4.93 to 477 pg/mL and 85.5 to 25,700 pg/mL respectively. Within-lab precision across the assay range was 3.2 to 18.1% for EDTA plasma and 4.0 to 16.5% for CSF. No significant interference was observed with endogenous blood components or 25 tested drugs including riluzole and edavarone. Serum and CSF levels demonstrated good result correlation with the QUANTERIX SIMOA assays with a correlation coefficient of 0.995 and 0.994, respectively. NfL was found to be stable in serum, plasma, and CSF for up to 6 freeze-thaw cycles, one week stored at room temperature, and one week refrigerated. Long term stability at -80°C was established for up to 2 years with studies ongoing.

Biogen used the Siemens Healthineers Atellica® NfL assay in clinical trials based on the assay's analytical performance and Siemens Healthineers' established support for clinical trials. Here we show data from VALOR, a Phase 3, placebo-controlled trial to evaluate tofersen in adults with SOD1-ALS over 28 weeks. NfL levels in tofersen-treated individuals reached their nadir by ~week 16, with a 65% reduction in CSF and 55% reduction in plasma at week 28. These reductions were maintained over time in the study's open-label extension (OLE). Placebo-treated individuals showed no reduction in plasma/CSF NfL levels in VALOR. Upon switching to tofersen treatment in the OLE, there was a clear reduction in CSF and plasma NfL levels in response to tofersen treatment after 28 weeks of treatment (55% reduction in CSF, 41% reduction in plasma).

Here we summarize the use of a Siemens Healthineers Atellica® NfL assay and its clinical utility in VALOR, a Phase 3 ALS study, demonstrating NfL is a useful biomarker in detecting a treatment response to tofersen in SOD1-ALS.

209. Analyzing the use of statins and survival in people living ALS using real-world data

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OBJECTIVE: To test if the usage of statins using real world data shows any impact on survival in people living with ALS who have or do not have a comorbidity of hyperlipidemia.

BACKGROUND: Natural history studies provide important information about disease progression for rare conditions such as ALS. Efficacy of statins on improving long-term health has been shown against heart disease and other harmful levels of LDL. However, studies of statins and ALS survival have had conflicting findings possibly related to difficulties with confounding by indication.

DESIGN: Mortality data was collected from 1,852 subjects from nine multidisciplinary ALS centers. Propensity score matching was used to make similar cohorts and recreate a hypothetical clinical trial with a group using statins and a group not using statins. We also ran secondary analysis to see if the results were different for patients with and without hyperlipidemia. The statin using and non-statin using groups were equally weighted with characteristics at enrollment such as El Escorial Criteria, ALSFSR-R, Vital Capacity, Sex, Region of onset, Diagnosis Delay and Disease Duration. Survival was defined as months between disease diagnosis and death, tracheostomy, or permanent assisted ventilation, whichever came first

RESULTS: The use of statins, in both patients with hyperlipidemia and regular lipid levels, did not show any differences in survival (p=.97 and p=.11 respectively)

CONCLUSIONS: According to our real-world data the usage of statin in ALS is not harmful. By using established statistical methods to find causality in observational studies, we avoided the need to recruit patients and establish a full clinical trial or biomarker study. While there could be a benefit to statin usage for specific patient populations, our initial holistic view into the real-world data supports the studies that found no impact of statin in ALS survival.

210. Arable land and vineyard proximity influenced ALS risk and age at onset

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Introduction

Amyotrophic lateral sclerosis (ALS) incidence and phenotypic heterogeneity is due to an interaction between genetic background and differently distributed environmental factors. We investigated the effect of living near specific croplands on ALS risk, site of onset, age at onset and progression rate.

Methods

In a population-based dataset of ALS patients (PARALS registry), diagnosed between 2007 and 2014, we recover the historical residence in the 20 years before disease onset. We gather data on the geographical distribution of croplands in the same period. For all the municipalities we calculated the percentages of area covered by each crop, comparing them to patients smoothed incidence using linear regression. Then, we calculated proximity scores by assessing the percentage of area covered by each crop enclosed in a circle centered on the residence address (radii range 100-2000 meters, Figure 1), using historical residence data, weighting each exposure by the residence period.

Results

ALS cases incidence increased according to the percentage of area covered in each municipality by arable crops, ranging from 0.75 to 1.81 cases/100.000/year ($R=0.191$, $p<0.001$, Figure 2). Using historical residential data, arable crops and vineyards proximity significantly influenced age at onset, even considering different radii (for arable lands 100-1500 meters, for vineyards 500-2000 meters) and stratifying for sex, site of onset and genetic status. We found no significant effect on progression rate or site of onset.

Conclusion

We confirmed a higher ALS risk in municipality with high percentage of arable crops. Arable crops and vineyards proximity significantly reduced median age at onset.

211. Associations between cognitive performance and glucose metabolism in ALS patients

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Background: Up to 50% of amyotrophic lateral sclerosis (ALS) patients have a cognitive impairment, but associations between changes in glucose metabolism as measured with ^{1}- F-Fluorodeoxyglucose positron emission tomography (FDG-PET) and patients' performance in various cognitive domains are yet to be examined in detail. Furthermore, the effect of partial volume effects correction (PVEC) on FDG-PET results has not yet been studied in ALS.

Aims: First, to replicate group-level differences in glucose metabolism between the following ALS groups: cognitively non-impaired patients (ALSni), cognitively impaired patients (ALSci) and cognitively and behaviorally impaired patients (ALScbi) in accordance with the revised Strong criteria. Second, to investigate associations between glucose metabolism and cognitive performance in various domains. Third, to examine the impact of PVEC of the FDG-PET data on the results.

Methods: Neuropsychological, clinical and imaging data from 68 ALS patients were analyzed. Cognitive performance was measured with the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), which assesses 5 cognitive domains, and with two social cognition tests. FDG-PET and structural MRI scans were acquired for each patient. All data were obtained at the Rostock site using the same MRI and PET scanners and with identical imaging protocols. We compared results between voxel-based statistical analyses carried out on non-corrected vs. PVE-corrected FDG-PET scans.

Results: ALScbi patients showed decreased signal in the right-superior and the middle-frontal gyri in addition to increased signal in the occipital areas compared to ALSni and ALSci patients. No differences were found between the ALSni and ALSci groups. A positive association was observed between patients' social cognition performance and their glucose metabolism in the right precentral gyrus. The results were largely unaffected by PVEC.

Conclusion: Group differences found in this study correspond with previous work in the field, supporting that cognitive and behavioral impairment is associated with widespread changes in glucose metabolism. Based on current findings, performance in most individual cognitive domains was not robustly correlated with glucose metabolism. This result may be related to the inherent limitation of ECAS being a screening test. PVEC does not seem to conspicuously affect the results, implying that the influence of atrophy is negligible in FDG-PET studies in ALS samples.

212. Autonomic Involvement in Spinal Muscular Atrophy: An exploratory study

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Background: Spinal muscular atrophy (SMA) is a neuromuscular disorder with progressive muscle weakness and atrophy characterized by degeneration of the anterior horn cells. The onset of weakness ranges from before birth to adulthood. Although emerging data suggests SMA is a multisystemic disease, autonomic involvement has not been widely investigated on adult SMA patients.

Methods: We evaluated the autonomic involvement in with 21 adult SMA patients with Sudoscan and COMPASS-31 and compared with age, gender, and body mass index (BMI) matched healthy controls. Other causes of autonomic disorders are excluded with appropriate analyses.

Results: Ten patients were female. Nineteen patients were type 3 and two were type 2. The mean age of the patients was 33.38 ± 10.12 (between 19 and 53). All patients were on nusinersen treatment. The mean Hammersmith Functional Motor Scale-Expanded (HFMSE) was 44.04 ± 19.05 (between 5 and 63). Six patients had three copies of SMN2, and fifteen had four. The mean COMPASS-31 score was 9.1 ± 9.8 (between 0 and 35), and there was no difference between patients and healthy controls. Regarding Sudoscan, electrochemical skin conductance (ESC) in hands and feet was similar between patients and healthy controls. Furthermore, no correlation was identified between ESC values in feet and disease severity measured by HMFSE, SMN2 copy number, or age. On the other hand, ESC values in hands correlated with HMFSE scores. Moreover, both type 2 patients showed markedly reduced ESC in hands.

Conclusion: Our study showed that ESC results and COMPASS-31 scores are not different in adult SMA patients from the healthy controls. Although previous studies suggested that autonomic involvement may be an underrecognized feature primarily in SMA type 1, of course further studies on larger cohorts are needed to confirm these results in type 2 and type 3 patients. On the other hand, decreased ESC in patients with lower HMFSE scores suggested that autonomic involvement may be a feature in patients with more severe disease course.

213. Availability of ALS medical care in Croatia and perception of the future treatments

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Introduction: The only ALS drug treatment currently approved in Croatia is riluzole. Non-invasive ventilation (NIV) and percutaneous endoscopic gastrostomy (PEG) are two treatments commonly used in ALS which have been shown to improve quality of life and survival. Our aim was to explore availability of ALS treatments at the level of local hospitals as well as physician perception of future ALS treatments

Methods: We created an electronic questionnaire that was distributed to the participants of the yearly national neuromuscular disorders symposium. Questionnaire inquired about physician demographics, level of training, availability of medical care for ALS patients at physician's place of practice and physician perception of the future ALS treatments

Results: We present first part of the collected data with final data to follow. So far 28 physicians have responded (71.4% women). Almost a third (32.1%) of physicians have said they are subspecialising in NMD, however majority (82.1%) reported treating less than 5 patients with ALS at the moment. Physicians who responded are equally distributed between large regional hospital centres and local general hospitals. Regional availability of ALS medical treatments is presented in Figure 1 with more than two thirds of hospitals providing all of them at the local level. Optimism regarding future treatment is presented in graph 1

Discussion: Due to very variable population density in different parts of the country with resulting differences in the number and sizes of hospitals we expected regional differences to be reflected in the availability of ALS medical care, especially regarding the procedures requiring hospital stays and interdisciplinary teams. However, PEG was a procedure all participants stated they could provide at the local hospital, while NIV was widely available with just a few hospitals having to refer to other more distant centre. Larger hospital centres unsurprisingly treated larger number of patients and had more NMD subspecialists, however, perception of the future of ALS treatment (in the light of new studies currently in progress) did not differ between physicians who remained neither optimistic or pessimistic

Conclusion: Recent advances in the treatment of neuromuscular disorders have not yet been reflected in the optimism of the neurologists treating ALS patients in Croatia, however it is encouraging that the currently approved ALS treatments are widely available at the local level\

214. Brain metabolism in symptomatic and asymptomatic C9orf72 repeat expansion carriers: a cross-sectional 1H and 31P magnetic resonance spectroscopy study

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Background

The onset and processes underlying neurodegeneration in amyotrophic lateral sclerosis (ALS) are largely unknown. We, therefore, studied brain metabolism in vivo in symptomatic and asymptomatic C9orf72 repeat expansion carriers.

Methods

We enrolled 15 asymptomatic carriers (fCO C9+) and 18 non-carrier family members (fCO C9-) of the C9orf72 mutation and 4 ALS patients with this mutation (ALS C9+). 2D proton (1H) and whole-brain phosphorus (31P) magnetic resonance spectroscopic imaging (MRSI) data were obtained using a 7 Tesla magnetic resonance (MR) scanner. After post-processing, fitting of the spectra and quality control, 12 brain metabolites (expressed as ratios), were compared between groups using weighted Bayesian linear multilevel models. Here, we summarize effects using maximum probability of effect (MPE, MPE>0.975 is comparable with p-value<0.05).

Findings

Compared to fCO C9-, fCO C9+ have decreased total N-acetyl aspartate/total creatine (tNAA/tCr) in the left precuneus (MPE=0.982) and decreased glutamate+glutamine/tNAA (Glx/tNAA) in the left precentral gyrus (MPE=0.998), but increased Glx/tNAA in the left precuneus (MPE=0.980) and right isthmus cingulate (MPE=0.995). Glycerolphosphoethanolamine/phosphocreatine (GPE/PCr) and uridine diphosphoglucose/PCr (UDPG/PCr) were increased in fCO C9+ as well, but increased UDPG/PCr was much more widespread in ALS C9+ than in fCO C9+ and ALS C9+ had also lower myo-inositol+glycine/tCr (mI+Gly/tCr) in the posteromedial regions and lower total adenosine triphosphate/PCr (tATP/PCr) in the parietal lobe (PME=0.984). Opposite to fCO C9+, ALS C9+ have likely increased Glx/tNAA in the left precentral gyrus (MPE=0.974).

Interpretation

fCO C9+ have signs of lower neuronal density (tNAA/tCr) and glutamate excitotoxicity (Glx/tNAA) in the precuneus but the primary motor region demonstrates resilience by keeping Glx/tNAA low, as opposed to ALS C9+. Increased membrane breakdown (GPE/PCr) was detectable in fCO C9+ and disturbed glycogen metabolism (UDPG/PCr). Glycogen metabolism is much more severely affected after phenoconversion (ALS C9+) and accompanied by decreased ATP and disturbed synthesis of membranes, intracellular second messenger system and (astro)glia (mI+Gly/tCr). We demonstrated these pathological changes of brain metabolism in symptomatic and asymptomatic subjects with a C9orf72 repeat expansion for the first time in vivo, which can have important therapeutic consequences.

215. C9orf72 in the Spanish ALS-FTD spectrum

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Background

Early detection of the C9orf72 mutation may allow for earlier diagnosis of these diseases, which could improve patient prognosis and treatment effectiveness. Analysis of C9orf72 can identify patients and families at higher risk of developing these diseases, which could help provide appropriate genetic counseling and preventive measures. Additionally, there are several ongoing clinical trials using antisense oligonucleotides (ASOs) to target the C9orf72 mutation in an attempt to reduce levels of the abnormal protein produced by the mutation.

Methods

1594 patients were studied and subdivided into 3 clinical groups: 1226 ALS patients, 201 ALS-FTD intermediate clinical patients, and 167 FTD patients. The methodology used for the analysis of the hexanucleotide repeat expansion in this patient series was triple-PCR, which allows for quantification of each repeat up to approximately 100 repeats, and conventional PCR, also called allele-specific PCR, which allows for the distinction of different lengths in the two alleles.

Results

The percentage of patients carrying the pathogenic expansion in C9orf72 in the ALS group was 4.5%, in the ALS-FTD group was 14.9%, and in the FTD group was 4.8%. Additionally, an increase in positive patients was observed among those with bulbar onset in the ALS group compared to those with spinal onset (<0.05). However, no differences were found in the number of expansion carriers between genders.

Discussion

As expected, the proportion of patients carrying the C9orf72 expansion is higher in groups with a family history within clinical subgroups, although it is not statistically significant (p>0.05). On the other hand, our cohort has typical characteristics of other previously published subpopulations, such as, for example, a 55-60% proportion of men, a 60-65% of patients with spinal onset, etc. Therefore, all the characteristics of other studied populations are found.

216. CARE-MND – A highly curated national integrated care, audit, and research platform for MND

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Introduction: The national motor neuron disease register for Scotland, CARE-MND (Clinical Audit Research and Evaluation of motor neuron disease), is a highly curated national integrated care, audit, and research platform with 99% case ascertainment, a high level of longitudinal clinical phenotypic data acquisition, linked bio-resourcing and high rates of autopsy. It was the first population-based register for people with MND (pwMND) globally at the time of launch in 1998. This study reports on outputs driven by the CARE-MND platform its relaunch in 2015 as an integrated digital platform for care and research, coinciding with a doubling in specialist MND nursing in Scotland.

Methods: We interrogated the CARE-MND database to ascertain the total number of pwMND registered, DNA samples acquired, range of observational and interventional studies supported, and number of brain and spinal cord donations.

Results: 2795 people have registered on CARE-MND since 2015. 471 are currently alive. CARE-MND has supported over 35 peer reviewed 'bench to bedside' publications including epidemiology, characterization of novel phenotypes, the impact of COVID-19 on survival, validation of novel clinical outcome measures, genomic studies, human iPSc and autopsy centred discovery science, and clinical trials. 1234 (44.2%) pwMND have consented to participate in research studies. 1062 (38.0%) have donated DNA samples to the Scottish Regenerative Tissue Bank. 613 (21.9%) have enrolled into observational studies and 221 (7.9%) have enrolled in the innovative definitive phase 3 MND-SMART trial.

Discussion: Co-production with pwMND, and close liaison with stakeholders including clinicians, laboratory researchers, and funders has enabled a high degree of participation in a range of inter-disciplinary research studies.

Conclusion: CARE-MND is a globally leading platform advancing research in a range of bench to bedside inter-disciplinary research, including epidemiology, genomics, , patient-reported outcome development, discovery science, participation in observational studies, and recruitment to definitive phase 3 clinical trials.c

217. Characterization of FTLD spectrum through advanced diffusion-weighted MRI metrics: a connectome approach

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Objective. To investigate structural alterations in brain network of FTLD spectrum using connectome analysis with advanced diffusion-weighted MRI metrics.

Materials. Thirty-four behavioral-variant frontotemporal dementia (bvFTD), 11 semantic variant primary progressive aphasia (svPPA), 11 nonfluent variant primary progressive aphasia (nfvPPA) and 18 motor neuron disease (MND) patients and 48 controls were enrolled and underwent multi-shell diffusion MRI. Fractional anisotropy (FA) maps were computed. Intra-cellular Volume Fraction (ICVF) maps were estimated using the NODDI model, providing a direct quantification of neurite integrity. Graph analysis and connectomics assessed global and local structural topological network properties and regional structural connectivity. In particular, mean distance (MD), eigenvector centrality (EC), degree centrality (DC) and sum of node weights (SN) metrics were extracted.

Results. Overall, widespread structural changes were observed in bvFTD patients relative to controls, MND and svPPA patients. nfvPPA patients showed altered FA properties (higher DC, SN and EC and lower MD) at global level compared to controls and MND patients. This condition was also verified at a lobar level, in particular in frontal, basal ganglia, parietal, and temporal areas. In addition, ICVF graph analysis measures showed that svPPA had a lower DC and SN in the temporal lobe compared to healthy controls and MND patients. Considering the regional connectivity analysis, bvFTD patients showed widespread decreased FA compared to MND patients and controls in all brain areas. In addition, bvFTD patients showed marked decreased FA strength relative to svPPA patients particularly in the right hemisphere, involving frontal lobe, supplementary motor area, putamen, parietal and temporal areas. nfvPPA patients showed a decreased FA in the left hemisphere relative to controls and MND patients, in particular involving precentral gyrus, supplementary motor area, insula, putamen and temporal lobe. Considering ICVF, greater alterations were detected compared to FA maps, showing differences also between svPPA and nfvPPA patients.

Conclusions. Connectome-analysis based on advanced diffusion-weighted models is useful to evaluate structural brain disruptions with greater differentiation among FTLD syndromes compared to diffusion-tensor derived measures.

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218. Characterization of the Enrolled Population in the Phase 3 PHOENIX Trial in Amyotrophic Lateral Sclerosis: Preliminary Results

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Background: AMX0035, an oral, fixed-dose combination of sodium phenylbutyrate (PB) and ursodoxicoltaurine (TURSO, also known as taurursodiol) significantly slowed functional decline and prolonged survival duration compared with placebo in adults with definite amyotrophic lateral sclerosis (ALS; revised El Escorial criteria), symptom onset ≤18 months, and baseline slow vital capacity (SVC) >60% in the phase 2 CENTAUR trial. The global phase 3 PHOENIX trial (NCT05021536; EudraCT 2021-000250-26) was designed to assess the efficacy and safety of PB&TURSO in a larger, more heterogeneous population of people living with ALS. Here, we report a preliminary profile of baseline characteristics for participants in PHOENIX conducted as of February 2, 2023, upon completion of trial enrollment.

Methods: Adults with clinically definite or clinically probable ALS (revised El Escorial criteria), symptom onset <24 months, and SVC ≥55% were enrolled from more than 65 sites, most of which are members of the Treatment Research Initiative to Cure ALS (TRICALS) and Northeast ALS Consortium (NEALS). Participants were randomized to receive PB&TURSO or matching placebo (3:2 ratio) by mouth or feeding tube for 48 weeks. Demographics and baseline disease characteristics were summarized using appropriate descriptive statistics. Categorical variables were summarized by counts and percentages of participants in corresponding categories. Continuous variables were summarized using mean (SD).

Results: A total of 664 participants with a mean (SD) age of 59.5 (10.81) years were enrolled from Europe (n=552) and the US (n=112). Most were male (62%) and identified as White (84%); 22% had bulbar-onset disease. Overall mean (SD) time since ALS symptom onset at screening was 14.4 (5.31) months; mean (SD) SVC was 82.8% (17.75%) and mean (SD) Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised total score was 36.7 (6.06) points at baseline. At screening, 92% and 3% of participants were receiving a stable regimen of riluzole and edaravone, respectively.

Conclusions: Summary baseline characteristics from the PHOENIX trial are presented; note that data are preliminary and subject to updates upon final database lock. Top-line efficacy and safety data from PHOENIX are anticipated in mid-2024.

219. Clinical and prognostic characterisation of patients with amyotrophic lateral sclerosis in Austria – a single-centre cohort study

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Background

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder associated with the predominant loss of upper and lower motor neuron function. Mean survival times are around 3 years after disease onset. A definite and accurate diagnosis can be challenging due to the substantial phenotypic and prognostic heterogeneity of the disease, which is important to consider in clinical routine as well as in clinical trials. Large cohort studies have the potential to identify clusters of ALS patients with specific clinical and prognostic features, but have so far not been performed in Austria.

Study aims

The objective of this study is to provide a clinical characterization of Austrian ALS patients and to assess the prognostic utility of clinical and laboratory parameters.

Methods

This single-centre, exploratory, retrospective cohort study included ALS patients treated at the Department of Neurology, Medical University of Neurology, between 01/01/2000 and 31/01/2023. Demographic, clinical and laboratory parameters were extracted from medical records. Disease progression was defined as the mean change of the ALSFRS-r (Amyotrophic Lateral Sclerosis Functional Rating Scale – revised) per month. Data on time of death were provided from Statistik Austria.

Results

A total of 222 ALS patients were analysed. Median age at disease onset was 62 years (IQR 52-70), and females accounted for 45% of the cohort. Median diagnostic delay was 10 months (IQR 5-18). Median survival time was 27 months (IQR 17-45, range 5-188 months). Younger age at disease onset and longer diagnostic delay were associated with longer survival ($p=0.001$ and $p<0.0001$, respectively). A spinal disease onset was present in 152 patients (68%), a bulbar/respiratory onset in 57 patients (26%), and 13 patients reported a combination of spinal and bulbar symptoms at disease onset. Survival did not differ significantly between patients with a spinal and bulbar onset. ALS variants were diagnosed in 15 patients (7%), with primary lateral sclerosis in 6 patients, progressive muscular atrophy in 5 patients and overlap syndromes in 4 patients (3 patients with ALS-FTD and one with ALS and Parkinson's disease). Genetic analyses were performed in 67 ALS patients (30%), out of which 5 patients (8%) were identified to have underlying pathogenic variants in the following genes: SOD1 (n=2), KIF5A (n=1), OPTN (n=1) and FUS (n=1). There was a significant correlation between neurofilament light chain (NfL) titres and the rate of ALSFRS-r change per month ($R2 = 0.4747$, $p = 0.0006$), as well as neurofilament heavy chain (NfH) titres and the rate of ALSFRS-r change per month ($R2 = 0.4401$, $p = 0.0134$). ALSFRS-r rate of change significantly correlated with survival time ($R2 = 0.6584$, $p = 0.0006$).

Conclusion

We present the first clinical and laboratory characterisation of a large cohort of Austrian ALS patients, confirming common prognostic parameters including age at onset, diagnostic delay and NfL as well as NfH. A bulbar onset, by contrast, was not significantly associated with shorter survival in our study. Further analyses will be performed to identify correlations between laboratory parameters and phenotypic clusters of ALS patients.

Conflict of interest

The authors declare no conflict of interest related to this work

220. Clinical description of C9Orf72-mutated ALS patients followed in a large and representative Center for Motor Neuron Diseases of central Italy

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Background

The expansion of a hexanucleotide repeat in C9Orf72 is associated with a wide spectrum of neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), Fronto-temporal Dementia (FTD) and Parkinson Disease. C9Orf72 is the most frequent cause of genetic ALS and is characterized by highly variable penetrance and pleiotropy. Therefore, affected members in a same family can show different phenotypes, and, in families where the gene has a low penetrance, it may cause disease' onset in elderly age, leading to a possible familiarity not always recognizable. This multifaceted behavior may also explain the challenges faced by gene therapies targeting this locus. Here, we report the characteristics of C9Orf7- mutated ALS patients in charge at our Center for Motor Neuron Diseases in Pisa, Italy.

Results

Eight patients with C9Orf72 mutation are currently followed at our Centre. They represent the 50% of patients with genetic ALS charged at our Centre, with a frequency in accordance with Literature data.

Six patients had a positive family history for neurodegenerative disorders and a relatively early disease onset (55 years old on average). Two of them had a familial positive history for both Motor Neuron Diseases (MND) and dementia; four had a familial positive history for isolated ALS; one had familial positive history for isolated dementia. Among the six patients, one had dementia, one had non-fluent aphasia and one had irritability at disease onset. They all developed a concomitant ALS. One patient developed isolated ALS without cognitive impairment. One of the patients developed a motor impairment consistent with Primary Lateral Sclerosis, with no cognitive impairment.

The remaining patients had a late disease onset (78 and 80 years old) and no familial track record for neurodegenerative disorders.

Conclusion

C9Orf72 is involved in many cellular processes, including vesicle trafficking, lysosome homeostasis, mTORC1 signaling and autophagy, though the precise functions of C9Orf72 is still unclear. This makes therapies targeting this gene especially challenging. Our report confirms that C9Orf72 mutations are associated with a wide phenotypic variability and variable penetrance, sometimes revealing unexpected positivities. The analysis of large panels of patients may increase our knowledge of pathogenic mechanisms of neurodegenerative disorders caused by C9Orf72 expansion.

221. CNM-Au8® Preserved ALS Patient Function and Slowed Disease Progression in the OLE of the Phase-2 RESCUE-ALS Trial: New Data Through to 120 Weeks

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Introduction

RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 in early sporadic ALS, followed by an ongoing open-label extension (OLE). CNM-Au8 is a suspension of catalytically active clean-surfaced gold nanocrystals shown to enhance neuronal metabolic energy and reduce oxidative stress.

Methods

Participants were randomized 1:1 to 30mg CNM-Au8 or placebo daily for 36-weeks during the double-blind period, followed by an OLE with all participants receiving 30mg CNM-Au8. RESCUE-ALS enrolled 45 participants [n=23 active, n=22 matched placebo]. Thirty-six participants continued into the OLE; 20 of 21 eligible participants (95%) originally randomized to CNM-Au8, and 16 of 19 eligible (84%) participants originally randomized to placebo. Current data represent a 12-month minimum follow-up from last-patient last-visit of the double-blind period.

Results

Post hoc analyses compared ALSFR-R slope change in participants originally randomized to CNM-Au8 versus placebo over two treatment periods (i) baseline to week 48, and (ii) week 60 to week 120 (when steady-state drug levels have been reached for ex-placebo participants). Significant differences were observed in: (i) ALSFRS-R slope from randomization to week 48 (p=0.0159), (ii) ALSFRS-R slope from week 60 to 120 (p=0.0057). Additionally, original randomization to CNM-Au8 delayed time to ALS clinical worsening defined as the first occurrence of death, tracheostomy, initiation of ventilatory support, or feeding tube insertion through 120 weeks (p=0.0494). CNM-Au8 treatment was well-tolerated with no significant safety findings reported.

Summary

These results demonstrate treatment with CNM-Au8 preserves functional status over longer periods of time, along with providing sustained treatment effects delaying ALS disease progression and prolonging survival.

222. Co-creation of new digital outcome measures in amyotrophic lateral sclerosis

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Background

Due to the rapid progressive characteristics of Amyotrophic Lateral Sclerosis (ALS) leading to severe disability, patients can encounter a burden to make on-site visits for routine medical care or to engage in clinical trials. Remote monitoring and the development of new digital outcome measures could help to overcome this, by facilitating remote monitoring, participation in clinical trials and meaningful outcome measures.

Aim(s)

To develop digital endpoints through understanding of the meaningful aspects of health (MAH), identification of concepts of interest (COI), and outcomes to be measured through a collaboration between Aparito's Patient Group Accelerator and ALS Liga Belgium. Using the Atom5TM software platform, we will test the usability and clinical utility and fit-for-purpose digital outcome measures.

Method(s)

A survey was conducted in partnership with ALS Liga Belgium with 16 ALS patients in France and Denmark, consisting of questions about daily impacts, symptoms, and acceptance of remote digital technology. A subsequent focus group provided further insight into the MAHs and COIs of ALS patients. A conceptual model was developed illustrating the MAH, COI, and outcomes to be measured, which will strengthen a fit-for-purpose software platform to capture outcomes of interest that will be further co-designed and tested for usability.

Results

Loss of independence and ability to carry out activities of daily living, alongside dependence on caregivers has a psychological impact on ALS patients. Fatigue, loss of motor function and muscle strength were reported as COI. Bulbar-onset patients reported the impacts of loss of speech on independence, which will be further investigated due to underrepresentation in this sample. Most respondents were confident in using an app to remotely capture their symptoms, with mixed responses regarding use of video assessments.

A fit-for-purpose Atom5™ platform for patient-generated data capture and analysis will be developed. The platform will be user-tested by ALS patients, who will provide feedback on the feasibility of performing and video recording tasks at home. A decentralized clinical study will be co-designed, evaluating the feasibility and potential clinical utility of the Atom5™ platform ('ALShome') and the clinical validation.

Conclusions

Capturing the aspects most relevant to ALS patients through co-creation is essential in the development of fit-for-purpose digital outcome measures

223. Coexistence of Amyotrophic Lateral Sclerosis and autoimmune diseases: two cases

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Background

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder involving upper and lower motor neurons. Myasthenia Gravis (MG) is an autoimmune disorder of the neuromuscular junction caused by antibodies against acetylcholine receptors in the postsynaptic membrane. Multiple Sclerosis is a potentially disabling autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination, gliosis, and neuronal loss. The exact ethiopathogenic mechanisms of ALS are still unknown, but among the several mechanisms proposed, immune system seems to be implicated in ALS pathogenesis, and several reports and nationwide registry studies have often confirmed association between the two.

Here we describe two patients affected from autoimmune neurological diseases who further developed ALS.

Cases

First case: a man affected from MG since 2013 who seven years later developed cramps, fasciculations, and left lower limb hyposthenia. All the exams ruled out a possible MG relapse, while neurophysiological and clinical assessment were consistent with ALS. Interestingly, he presented SOX1 antibodies, known to cause paraneoplastic damage of neuromuscular junction and dysimmune neuropathies not related to cancer. However, an accurate paraneoplastic screening resulted negative. During the neurological follow-ups, the patient showed a disease progression with a deterioration of the clinical picture.

Second case: a 70 years old man affected by clinically stable multiple sclerosis since several years, who developed a rapidly progressive neck hyposthenia with head drop, diffuse muscle hypotrophy and diffuse fasciculations. A complete virological, rheumatological, and paraneoplastic screening resulted negative, while an electromyogram showed neurogenic pattern with acute denervation in all test muscles. High camp brain and spine MRI did not show any changes in the white matter lesions, while revealed a hypointensity in primary motor cortex consistent with upper motor neuron degeneration.

Conclusion

Several large population studies reported a higher incidence of autoimmune diseases in ALS patients, though is unclear whether a causal relationship exists. Dysfunctional immune regulation and increased inflammatory responses in both autoimmune diseases and ALS may explain the coexistence of ALS and autoimmune diseases and may be a predisposing factor for ALS development in predisposed patients.

224. Coexistence of Amyotrophic Lateral Sclerosis and Pseudoxanthoma Elasticum in two siblings

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Background

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder involving upper and lower motor neurons. The exact ethiopathogenic mechanisms are partially unknown, though a genetic predisposition is thought to be pivotal in both familiar and in sporadic cases. Among the pathogenic mechanisms proposed, several studies have disclosed a possible correlation between angiogenesis and ALS, since mutations in the ANG gene for angiogenin are considered a risk factor for developing ALS, and lower VEGF levels have been found in ALS.

Pseudoxanthoma elasticum is a rare genetic disease characterized by a progressive ectopic mineralization of elastic fibers in the skin, retina and blood vessels. Individuals most commonly show angioid streaks of the retina with retinal hemorrhage and/or characteristic papules in the skin. This disease is mostly related to a mutation in ABCC6 gene, which is involved in the homoeostasis of serum pyrophosphate (PPi), a main inhibitor of ectopic calcification. Dysfunctional or missing ABCC6 results in low serum PPi and, thus, in ectopic calcification, especially of soft connective tissue.

As far as we know, there are no cases of coexistence of ALS and PXE described in Literature. Here we describe two siblings affected from PXE who also developed ALS.

Case

The woman, who was a healthy carrier for PXE and did not show any clinical manifestations of PXE, developed at the age of 60 a rapidly progressive ALS. She died one year after the disease onset. Her brother, affected from clinically manifested PXE from the young adulthood, developed a slight dysarthria at the age of 68. He performed an electromyogram showing neurogenic pattern with fasciculation potentials in all test muscled. A high camp brain MRI revealed hypointensity in primary motor cortex, consistent with upper motor neuron degeneration. The patient slowly developed head drop, dysphagia and sight limbs hyposthenia, but after 2 years, he is still ambulant. Interestingly, genetic test for ALS-related genes resulted negative.

Conclusion

Although alteration in angiogenesis are supposed to play a role in pathogenesis of ALS, there is no data on a possible role of PXE in relation to ALS development. We have no explication on why the brother most affected from PXE developed a less aggressive form of ALS, however description of cases like this in object may contribute to the discussion around ALS pathogenesis.

225. Colchicine treatment in Amyotrophic Lateral Sclerosis: a phase 2 multicenter, randomized, controlled, double-blind clinical trial

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BACKGROUND

In preclinical studies colchicine, besides its anti-inflammatory action, was found to enhance the expression of autophagy players and blocked TDP-43 accumulation, a hallmark of ALS. Colchicine has never been tested on ALS patients.

METHODS

In this multicenter, randomized, double-blind trial, probable or definite ALS patients with symptoms onset within 18 months, were randomly assigned in a 1:1:1 ratio to receive colchicine 0.01 mg/Kg/day, 0.005 mg/Kg/day or placebo for 30 weeks. The primary outcome was the number of patients exhibiting a decrease fewer than 4 points in the ALSFRS-R total score from baseline to treatment end. Secondary outcomes included the changes from baseline of the autophagy pathway, stress granule response, TDP-43 intracellular accumulation, extracellular vesicles secretion and neurofilaments, comparing colchicine and placebo arm. Clinical outcome measures of disease progression, survival, safety and quality of life were also collected.

FINDINGS

After screening 57 persons, 54 were randomly assigned to colchicine or placebo. In ITT analysis 35.7% of patients treated with the lower dose of colchicine (0.005 mg/kg/day) versus 13.3% with placebo had a decline fewer than 4 points

at ALSFRS-R in 30 weeks (p=0.208).

While before treatment there were not differences in ALSFRS-R monthly variation between colchicine and placebo arms (mean -0·025, 95%CI: -0·31 to-0·26), there was a slower decline only in patients treated with colchicine 0.005 mg/kg/d during treatment compared to those who received the placebo (mean difference 0·53, 95%CI: -0·13 to 0·93, during treatment, p=0.011; mean difference 0·46, 95% CI: 0·12 to 0·79, after treatment, 0p=0.010). Colchicine induced the expression of HSP70 in peripheral mononuclear blood cells of treated patients compared to controls and decreased insoluble TDP43. The total number of reported adverse events (AEs) was 14 for placebo arm, and 34 for the colchicine arms. Severe AEs were 6 in the placebo group, and 12 in the colchicine groups. Events occurring at a greater frequency in the treated group were gastrointestinal disorders, injuries, metabolism and nutrition disorders, skin and psychiatric disorders.

Based on clinical/biological effects and safety, colchicine 0.005 mg/kg/day resulted the best dosage in this study.

INTERPRETATION

A treatment of 30 weeks with colchicine was safe, but further trials are necessary to evaluate efficacy of this already available drug in ALS.

226. Combining patient preferences, function and survival in clinical trials for amyotrophic lateral sclerosis

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Background: Mortality plays a pivotal role in the assessment of therapeutic interventions for Amyotrophic Lateral Sclerosis (ALS). Here we expand the Patient-Ranked Order of Function (PROOF) to weigh the improvement in symptoms according to patient preferences and their overall survival time.

Methods: An online survey was sent out to a population-based registry in the Netherlands at baseline and after 12 months. Patients were asked to score functional domains and rank the order of importance of each domain. Vital status was extracted from a municipal population register. We integrated PROOF with the Combined Assessment of Survival and Function (CAFS), and derived a composite that includes functional domains, patient preferences and survival time. We illustrate its use in a hypothetical trial and present alternative metrics to facilitate its interpretation.

Results: In total, we included 430 patients with ALS with complete survival data. Patient preferences at baseline were associated with survival, especially with the most bothersome domain (log-rank $p < 0.001$). Using change from baseline led to paradoxical ranks at 12 months, where patients with poor baseline scores could be ranked best at the end of follow-up; this was resolved by using observed scores. We introduce the win probability, win odds and number needed to treat as metrics for group comparisons and illustrate how they are affected by incorporating patient preferences.

Conclusions: Combining patient preferences, functional outcomes and survival time into a single metric provides a balanced overview of the totality of evidence and could refine the risk-benefit assessment of new therapeutic interventions.

227. Correlation of cardiac Troponin T with parameters of body plethysmography, polysomnography and diaphragmatic ultrasound in patients with Amyotrophic L

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Introduction and Background:

One of the most devastating consequences of Amyotrophic lateral sclerosis is respiratory muscle weakness and subsequent respiratory failure.

Cardiac Troponins T (cTnT) and I (cTnI) are highly specific biomarkers of acute myocardial injury. However, diseased skeletal muscle can be a source of cardiac Troponin T in chronic skeletal muscle disorders (1).

In ALS, cardiac TnT is elevated in > 60% of patients. It correlates with disease severity and was therefore suggested as a biomarker (2). Our aim was to further investigate the relation of cTnT to disease progression. We postulated that increasing cTnT levels are related to respiratory muscle impairment.

Methods:

In this retrospective cross-sectional study, we reviewed the real-world cohort from our amyotrophic lateral sclerosis clinic at the University hospital Bonn to identify ALS patients with results of respiratory function tests in between March 2019 and December 2021. We incorporated 66 results of body plethysmography, 17 polysomnographic test results and 41 results of diaphragmatic ultrasound. Hs-cTnT, cTnI from serum and pNfH from liquor was available as part of routine diagnostic procedure. Non-spearman correlation and simple linear regression were used to investigate the relationship of respiratory parameters with cTnT.

Results:

We included 92 patients with a mean age of 62 years, out of which 38 % were female. Cardiac Troponin T was increased in 61% of ALS patients. The mean cTnT was 25,94 ng/ml (± 30,89), while cTnI was 5,92 (±3,74).

In body plethysmograph results FVC% showed the best negative correlation (rs=-0,5251, p<0,0001) followed by VC % (rs=-0,5197, p<0,0001) and FEV1% (rs=-0,4191; p=0,0006).

In contrast, the parameters did not correlate with Neurofilament (pNfH). In ROC analysis, cTnT shows a good diagnostic accuracy in distinguishing FVC values below 70% (AUC 0,77; p=0,0012). Polysomnographic and diaphragmatic ultrasound measurements did not show significant correlations with cTnT.

Conclusion:

This study suggests that increased cardiac Troponin T levels are negatively correlated with respiratory function in ALS. Progressive respiratory muscle weakness might be a specific source of cTnT. Further research is necessary to understand underlying pathophysiology and dynamics of cTnT elevation. Potentially cTnT might serve as a surrogate marker to hallmark stages of respiratory failure.

1: (du Fay de Lavallaz et al., 2022)

2: (Castro-Gomez et al., 2021)

228. Course of cognitive and behavioral impairment, quality of life and caregiver status in Amyotrophic Lateral Sclerosis (ALS): A 6-Month Follow-Up Study

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BACKGROUND: ALS is associated with cognitive impairment (CI) characterized by alterations in phonemic verbal fluency, language, executive functions (EF), theory of mind (ToM), and verbal memory, as well as behavioral traits like apathy. However, the course of cognitive and behavioral impairment (CBI) in ALS is unclear. We hypothesized a worsening in patients' self-reported quality of life (QoL) and an increased caregiver burden over time.

OBJECTIVES: Our aim is to investigate changes in CBI, QoL and caregiver status in an ALS cohort at 6-month follow-up period.

METHOD: We assessed 17 patients with ALS and their primary caregivers using an extensive neuropsychological battery, including Edinburgh Cognitive-Behavioural ALS Screen (ECAS) versions A and B, other specific tests for cognitive domains (language, EF and ToM), affective symptomatology and behavioral scales, Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-40), caregiver's questionnaires and Revised ALS functional rating scale (ALSFRS-R). We compared paired measures in the ALS cognitive and behavioral impairment (ALSCI/CBI) and ALS behavioral impairment (ALSBI) groups using Wilcoxon test.

RESULTS: Mean age and years of education were 64.4 ±11.8 and 8.8 ±4.2 respectively, 41.2% were women, 76.5% had limb onset and mean of baseline ALSFRS-R was 35.35 ±7.35. Based on Strong criteria, 47.1% were ALSCI/CBI and 52.9% were ALSBI (76% apathy). After 6 months, the ALSBI group showed a better performance on the ECAS-EF ($z=-2.31$, $p=0.02$), while the ALSCI/CBI group surprisingly showed a significant reduction in the number of errors on the Wisconsin Card Sorting Test ($z=-2.20$, $p=0.03$). Additionally, the ALSCI/CBI group had a higher score on the ALSAQ-40 ($z=-2.0$, $p=0.04$). No significant differences were observed in other cognitive tests, behavioral, anxiety and depression scales, or caregiver emotional distress and burden questionnaires in either group.

CONCLUSION: Our preliminary findings suggest that cognitive, behavioral, and caregiver status remain stable in an ALS cohort for a period of 6 months, regardless of their baseline CI. Interestingly, we observed better performance in some EF tests in both groups, which could be related to a practice effect. However, there is a lack of longitudinal studies exploring how ALS patients differ from normal age-related cognitive and behavioral decline. Furthermore, we observed that the decline in perceived QoL was more noticeable in patients with baseline CI.

229. Crushing riluzole tablet: methodology of a home-simulation experiment to evaluate the loss of powder and active principle in this product manipulation

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Difficulty in swallowing oral formulation has been documented in dysphagic and non-dysphagic people; in amyotrophic lateral sclerosis (ALS) patients it can compromise the management of oral treatments and may induce them to crush the tablets, outside the labeling instructions, and potentially expose them to several risks. Negative effects of crushing tablets, e.g. loss of powder and API, were documented by research performed in professional settings (researchers or nurses using specific tools). Considering that ALS patients and/or their caregivers often crush tablets at home, even without experience in drug preparation or in the use of professional tools, we set up a simulation experiment to evaluate the impact of crushing riluzole tablets on loss of powder and API.

The crushing tests were carried out by 15 healthy volunteers, without any professional in the preparation of medication. No other selection criteria were applied. Crushing professional devices were not considered in this study since not commonly used in the outpatient setting that we wanted to simulate. A total of 150 coated tablets, obtained from 3 different packs of riluzole were used, each volunteer was asked to manually crush 5 tablets for each method: method A – crushing the tablets, directly on the table or on paper, with a meat tenderizer - the powder was collected through paper or by sliding a knife across the table and transferred to volumetric flasks; method B crushing the tablet between two spoons - the powder was collected in the lower spoon and directly transferred to a volumetric flask. Tablets before crushing and the powder obtained after crushing were weighed and the residue (loss) of powder was calculated as a percentage of the intact tablet weight. Moreover, the powder of each tablet was diluted and analyzed by HPLC: the loss of API as a percentage of the labeled content (i.e. 50 mg) was calculated. Mean and Standard deviation were calculated across all methods used and for each method individually.

This is the first study on the impact of crushing the riluzole tablets on the loss of powder and API in a simulation of a real-world contest. Considering the relation between API, daily dosage, and effectiveness of riluzole, this experimental research will provide evidence about the consequences of this unauthorized manipulation and help neurologists to take an informed decision about the best formulation of riluzole for ALS patients.

230. Decision making about gastrostomy placement in MND: a survey of UK healthcare professionals beliefs and practice

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Background: Healthcare professionals (HCPs) practicing within multidisciplinary teams (MDT's) play a crucial role helping people with MND (pwMND) to make decisions about gastrostomy placement. The aim of this study was to understand the beliefs and practice of UK HCPs about supporting pwMND making decisions about gastrostomy placement and identify any differences between the professional roles involved.

Methods: HCPs with experience of supporting pwMND to make decisions about gastrostomy placement were recruited to an online survey 13/6/2022-30/8/2022. Descriptive and comparative statistics were used to analyse the data.

Results: A total of 139 participants from a range of healthcare professions with a mean 11 years' experience of caring for pwMND completed the survey. Dysphagia (99%) and weight loss (91%) prompted participants to discuss gastrostomy placement with pwMND. While 64% of participants believed they initiated discussions with pwMND about gastrostomy at the right time, 36% believed gastrostomy placement should ideally be introduced to pwMND earlier in the disease course than they initiated such discussions in practice. More participants discussed the risk of aspiration (22% v 11%; p 0.018), choking (27% v 8%; p 0.0002) and prognosis (17% v 3%; p 0.0002) in relation to declining gastrostomy compared to if discussing commencing the intervention. Prognosis was not discussed in relation to commencing or declining gastrostomy placement by 61% of participants. Half (48%) of participant's believed gastrostomies were placed too late. While 52% of participants believed that HCPs should give pwMND recommendations about gastrostomy placement, participants were more likely to 'often' or 'always' recommend pwMND to have a gastrostomy placed (23%), than to continue without (7%) or decline the intervention (4%) ($p<0.001$). Significant differences between the responses of different professions were identified with nurses and dietitians discussing the broadest range of issues with pwMND, and doctors being more likely to address prognosis and procedural mortality risk.

Conclusion: This study has implications for how healthcare teams organise and deliver decision support to pwMND. MND MDT's may need to establish a consistent cross-profession approach to delivering decision support, to facilitate pwMND understanding of all options available. Further research is required to identify and overcome any barriers to timely decision making about gastrostomy placement.

231. Decision-making about gastrostomy placement and ventilation in motor neuron disease (MND) care: a qualitative evidence synthesis

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Background: People with MND (pwMND) make decisions about whether to accept interventions in the complex context of a progressive neurological disease without a cure, following interactions with multiple external agents including caregivers and healthcare professionals (HCPs). The aim of this review was to synthesis the findings from the published qualitative literature that explored the perceptions of pwMND, their caregivers and HCPs when making decisions about gastrostomy placement or ventilation (non-invasive or invasive).

Methods: A comprehensive and systematic bibliographic and supplementary search strategy identified 27 papers that met the inclusion criteria. The data that related to pwMND, caregivers or HCPs perspectives of making intervention decisions were extracted and included in the thematic synthesis which was managed using the NVivo qualitative data analysis software.

Findings: The prospect of commencing an intervention generated an emotional response in pwMND, including fear or anxiety about the procedure or living with the intervention. This included pwMND were concern for the quality of any extension to their lives and the impact of the interventions on their caregivers. Caregivers and HCPs were intricately involved in the process of decision making. Whilst caregivers and HCPs might help pwMND understand the interventions and their options, there was evidence that pwMND were influenced by their views. The timing of discussions about interventions and when they should be commenced was a source of uncertainty for pwMND and concern for HCPs. Worsening symptoms and guidance from HCPs were factors that led to pwMND to consider an intervention.

Conclusion: pwMND decisions about interventions were made within the context of a disease without a cure, uncertainty about the rate of disease progression and multiple interactions with their caregivers and HCPs. pwMND emotional response to the consequences of interventions and the impact of dialogue with others on pwMND autonomy and sense of agency influenced decisions about whether and when to start interventions. Further prospective research is required to understand how to best support pwMND to make informed, autonomous, timely decisions within this complex context.

232. DESIGN OF AN INTERNATIONAL, PHASE 3 OLE STUDY TO INVESTIGATE THE LONG-TERM SAFETY OF DAILY ORAL EDARAVONE (FNP-122) IN ALS: THE ADOREXT STUDY

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Objective: To describe the design of the ADOREXT study, a Phase 3 OLE study trial, assessing the long-term safety of once-daily oral edaravone (FNP-122) in people living with ALS (pALS).

Introduction: Effective treatment of ALS continues to be a significant unmet need. Edaravone is thought to be a potent antioxidant and may slow the progression of ALS by protecting nerve damage.

All sites that recruited pALS for the pivotal phase 3 ADORE study, most of them from the TRICALS Network, can participate in the OLE study ADOREXT.

Methods: The ADOREXT study offers to all ADORE participants the possibility of receiving FNP-122 after they complete the full study period. pALS that discontinued treatment in the main ADORE study for other than safety reasons, will be also invited to participate in the ADOREXT to also receive FNP-122. Riluzole may be used as background (add-on) therapy.

The main inclusion criteria is all pALS who completed the full study period in the ADORE study and whom the investigator has no concern and judges tolerable for initiating or continuing treatment with FNP-122 from a risk and benefit point of view.

The primary endpoint is the nature, frequency, and severity of TEAEs in pALS.

Secondary endpoints include mortality-adjusted change from baseline in ALSFRS-R total score until the end of the study, survival time, change from baseline in SVC, change from baseline in the prognostic ALS biomarkers (neurofilament light, creatinine, creatinine kinase, 8-OHdG and Urinary p75ECD, PROs (ALSAQ-40 and EQ-5D-5L), VAS and ECAS.

The ADOREXT protocol has been developed considering pALS's needs and perspectives collected in an ALS Patient Representatives Advisory Board (PAB) in which 7 patient organizations from Europe and North America participated. The aim was to appraise the patients' perception on the clinical protocol to improve accessibility of the trial to patients as well as to validate endpoints and study design.

Results: Inputs and experiences on pALS Needs & Expectations, Study Design, Treatment, Hospital Visits, Safety Reporting and Questionnaires were collected during the PAB and were considered in the ADOREXT trial design.

Roll over from the ADORE study to the ADOREXT study starts in March 2023.

Conclusions: The ADOREXT study will provide relevant safety and long-term survival data on this daily oral formulation of edaravone.

233. Designing a neurophysiological module for multicenter early phase clinical trials in people with ALS

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Introduction: A frequently observed mechanism in rodent and iPSC-derived models of amyotrophic lateral sclerosis (ALS), as well as in patients living with ALS, is changes in motor neuron excitability. Altered excitability have been suggested to represent early pathophysiological mechanisms associated with motor neuron death, and have been associated with more aggressive disease and shorter survival. Compounds that target membrane excitability may therefore hold disease-modifying potential. State-of-the art neurophysiological techniques, such as motor unit number estimate methods and nerve excitability testing, allow for early identification of motor unit loss and changes in nerve excitability in ALS, and could improve timely detection of relevant therapeutic effects. With preclinical evidence of compounds that modulate motor neuron excitability (e.g. QRL-101), axonal excitability measures have the potential to serve as translational tools to validate target engagement.

Methods: A neurophysiological module will be designed as part of an early phase multicenter study (QRL-101-02). Within this module, nerve excitability testing will form an integral component. Excitability recordings will be performed on the median nerve at the wrist and motor responses will be obtained from the abductor pollicis brevis. Consecutive recordings will be performed over time in approximately 24 ALS patients.

Results: Markers of motor axonal excitability will serve as exploratory pharmacodynamic endpoints to detect effects of the studied compound (i.e. QRL-101) on (changes in) ion channel activity and axonal membrane properties. These findings will also be related to disease state in terms of axon loss.

Conclusion: The results of the current study will provide highly relevant information on measures of axonal excitability as pharmacodynamical markers. This study will also offer input for further optimization of neurophysiological techniques in a multicenter setting, facilitating widespread adoption for future clinical trials, and ultimately contribute to, and guide, early phase ALS drug development.

234. Developing a Remote stimulation-free Motor Unit Number Estimate (REMUNE).

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Background: Motor Neurone Disease (MND) affects the motor neurones responsible for transmitting signals to the muscles to produce voluntary movements. The current muscle activity recording equipment (Motor Unit Number Estimate (MUNE)) involves electrical stimulations, which can induce discomfort in some participants, to accurately estimate the number of functional motor units and monitor the disease progression. These assessments also require individuals with MND to come to a clinical-based environment periodically. Developing a stimulation-free MUNE would allow a greater amount of data collection as the assessments would be more comfortable, no longer require specialist staff and be less time-consuming. Overall, it is a first step in making these assessments remote and home-based in the future.

Methods: People with MND (n≥24), and healthy age-matched controls (n≥12) will attend six assessments over 12-months. During these assessments, 64-channels surface EMG sensors will be applied bilaterally over the thumb, index finger, little finger, and shin for a duration of 16-minutes. While recording their muscle activity, the participants will perform several muscle contractions at different force levels. Afterwards, for the same muscles, MUNIX, a gold-standard MUNE, will be performed as a comparative measure of motor unit loss.

Objectives:

• To reliably identify the active motor unit pool at varying force levels by combining HD-sEMG with an advanced motor unit decomposition technique.

• To seek physiological correlations between motor unit parameters, muscle power and MUNIX over time and across body regions in MND and control patients.

• To define a novel stimulation-free MUNE.

So far, the methodology has been performed on three volunteers, data has been collected from 24 muscles in total amounting to 48-minutes of recording.

235. Developing and evaluating complex digital interventions for people with MND: A case study of the Telehealth in MND service.

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Complex interventions include a variety of different components, target multiple behaviours, and are delivered by several healthcare professionals (HCPs) to people across a range of conditions. The care for people with Motor Neuron Disease (pwMND) and delivering digital healthcare services are complex interventions. These require specialised development and evaluation methodologies, which looks beyond assessing a singular outcome using a randomised control trial to research that explores what works, for whom and why.

We have developed a remote monitoring system called Telehealth in MND (TiM) on MyPathway that aims to facilitate communication between HCPs, pwMND, and their carers. Through TiM, pwMND and their carers complete regularly scheduled symptom questionnaires, answers to which are securely sent to a clinical dashboard, enabling HCPs to make treatment decisions or monitor progress. Through multiple, iterative studies we have continued our co-design, person-based approach and begun to evaluate this complex intervention alongside implementing it into healthcare services. The TiM system has successfully received multiple rounds of funding for development, evaluation and implementation from charity and national organisations.

Using TiM as a case study, we share our learning of using a co-design approach to develop this complex system from an initial research prototype to implementation in multiple UK and European MND centres as a clinical service. This includes the adoption of a person-based approach and interviews and focus groups with a variety of users, to ensure the system meets their needs. As part of a process evaluation, a variety of important real-world data were collected to continually develop the service. Through these mixed methods and iterative development cycles, the TiM system has been adapted to meet the needs of users (pwMND, their carers, and HCPs) at care centres, collected over 14,000 outcome measures, been shown to be acceptable to users, with 85% of regular questionnaires being completed, which rises to 98% for longer-term users.

We will discuss the challenges of system implementation, which are often neglected and seldom reported in the research literature. We anticipate that this will be of interest to anyone developing, evaluating, or implementing their own digital health service or complex intervention to support pwMND or other related neurological conditions.

236. Diagnosis and symptom characteristics of diverse patient populations with amyotrophic lateral sclerosis in the USA

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Amyotrophic Lateral Sclerosis (ALS) symptom onset is gradual and includes muscle weakness or stiffness, dysarthria, dysphagia, and respiratory difficulties. The objective of this study is to further understand the diagnosis of limb and bulbar symptoms leading up to ALS diagnosis in the USA, examining variations by sex and race.

This retrospective cohort study was conducted using the Optum® Market Clarity database, which contains electronic health records (EHR) and insurance claims records. Cases had their first diagnosis for ALS between January 2015 and December 2019. Cases had either one ICD-9/10 code in EHR or two ICD-9/10 codes ≥30 days apart in insurance claims during the study period. All cases were required to have ≥5 years of enrollment in the database prior to first diagnosis. Descriptive statistics were calculated stratified by sex and race/ethnic groups.

This study identified 3962 individuals with ALS, of whom 1820 were female (46%) and 2142 were male (54%). Most individuals identified as Non-Hispanic White (71.3%), followed by African American (6.0%), Hispanic (3.0%), and Asian (0.8%), with missing race/ethnicity for 18.9%. At any time in the 5-year period before ALS diagnosis, 41.9% of subjects were diagnosed with at least one bulbar symptom, 77.3% with at least one limb symptom, and 34.5% with both bulbar and limb symptoms. Females were diagnosed more frequently than men: any bulbar 45.3% vs 39.0%, (P<0.0001), any limb 79.0% vs 75.8%, (P=0.02), both 37.6% vs 31.8%, (P=0.0002). Limb symptoms occurred more frequently among individuals identifying as African American (85.7%), followed by Non-Hispanic White (78.1%), Hispanic (76.7%), and Asian (70.6%). However, no differences in diagnosis of bulbar symptoms (P=0.17) or both bulbar and limb symptoms (P=0.18) by race/ethnicity were present. When increasing the sample size to include individuals with only 1-year prior to ALS diagnosis (N=7932), the findings were similar.

In our study population ALS was present in more males, but women were diagnosed more frequently with bulbar and limb symptoms. African Americans tended to have more limb diagnoses than other race/ethnicities. There were no racial/ethnic differences in bulbar symptoms. Future research into the occurrence and timing of other symptoms' diagnosis by sex and race/ethnicity prior to ALS diagnosis will be valuable in further understanding the patient population and the ability for more timely diagnoses.

237. Digital outcome measure assessing the motor function of patients with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of motor neuron leading to weakness and death often resulting from failure of the respiratory muscles. There have been numerous recent discoveries in ALS research leading to potential therapies that will need testing in clinical trials. Due to limitations of traditional outcome measures, trials require large sample sizes, long durations. Recently, a pivotal ALS trial fails to meet its primary target and researchers attribute it to the endpoint selection rather to the drug product.

Digital biomarkers collected with wearable devices provide an objective assessment performed in real-life and offer powerful alternatives to traditional outcome. SV95C is the first digital outcome measure qualified by EMA for ambulant patients living with Duchenne Muscular Disease. It demonstrates that digital outcome can measure remotely and reliably motor function and have the potential to enable shorter trials with less patients.

We designed a longitudinal natural history study in ALS and tested several candidate digital outcome measures. Participants were assessed with standard evaluations including the amyotrophic lateral sclerosis functional rating scale, six-minute walk test, Medical Research Council scores, Ashworth score, hand dynamometer, pulmonary function and cognitive tests every 3 months for 1 year. After each visit, patients were asked to wear for one month one inertial sensor at the ankle and one at the wrist for continuous assessment of motor function in real-life. These data will be compared with already acquired normative data. At the present time, 11 patients with ALS (3 females, 8 males) aged from 44 to 72 years old were included. Four patients discontinued the study due to death or permanent ventilation.

We will present performance of the candidate digital outcome measures and compare the most robust digital measure to traditional outcome to test its capability to enable more efficient trials.

238. Directly measuring network function during social cognition in ALS using EEG during the Reading the Mind in the Eyes Task

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Background: Deficits in recognition of emotions is part of the cognitive impairment which occurs in ALS. Such symptomatic changes in social cognition can be quantified by the Reading the Mind in the Eyes Task (RMET)[1]. However, performance in this task may not capture early or subtle impairments in social cognition, despite underlying pathophysiology. It has previously been demonstrated that, during RMET performance, cortical network engagement during determination of state of mind can be captured by EEG in the form of an N270-400 wave[2].

Objectives: To determine if dysfunction in cortical networks driving social cognition can be directly captured and quantified in ALS using EEG by examining the N270-400 wave.

Methods: A modified version of the RMET is performed during recording of 128-channel EEG. The average cortical activation (event related potential, ERP) which occurs during correct recognition of individuals' emotional state is compared to that captured during recognition individuals' sex, as a non-social control. Recruitment is ongoing, with datasets from 13 controls and 21 people with ALS collected to date. A neuropsychological test battery was also undertaken with 7 controls and 10 people with ALS.

Results: An N270-N400 is evident over the right inferior frontal cortex across controls and people with ALS. Analysis of data collected to date indicates that the N270-400 ERP occurs earlier (has shorter latency) in those with ALS ($p=0.047$) as a group. Correlation analysis including all participants indicates that those with larger N270-400 waves (more negative mean amplitude and area) have better semantic verbal fluency (animal naming test verbal fluency index, $p=0.012$-0.016, $rho=0.62$-0.64). No significant correlation was found between this wave and RMET performance or other test battery measures of executive function, memory or behavioural inhibition.

Discussion: The RMET-associated N270-N400 waveform is evoked in both controls and people with ALS, however as size or delay of the wave does not correlate with RMET performance, but does correlate with semantic fluency, this EEG measure may reflect the function of language or executive networks during the task, rather than that of networks specifically involved in social cognition.

1. Burke et al. PLoS One. 2016; 11(8):e0160850

2. Sabbagh et al. Journal of cognitive neuroscience. 2004; 16(3):415-426

239. Double trouble: clinical and neuroimaging features in a case of frontotemporal dementia with C9orf72 expansion and progranulin mutation

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Introduction. Clinical and MRI features of a patient with behavioral variant frontotemporal dementia (bvFTD) carrying a double mutation in the progranulin (GRN) and C9orf72 genes are described.

Methods. The proband was matched with eight FTD patients carrying a C9orf72 expansion and eight carrying a GRN mutation. Sixteen age- and sex-matched healthy controls were also included. Subjects underwent a 3T MRI T1 scan and a full neurological and cognitive evaluation. Voxel-based morphometry was performed. Gray matter volumes of subcortical, hippocampal and cerebellar structures were also obtained. MRI features were compared between the proband and other groups.

Results. The proband was a 71-year-old male presenting with behavioral derangement and language impairment. Compared to controls, he presented atrophy of bilateral fronto-temporal cortex, mainly left-lateralized, and bilateral posterior cerebellum. C9orf72 patients showed bilateral frontotemporal involvement, while GRN patients mainly presented left-sided atrophy. The proband showed subcortical atrophy of bilateral caudate, right hippocampus and bilateral thalamus compared to controls, similar to that expressed by C9orf72 patients (left caudate and bilateral thalamus) and GRN patients (bilateral dentate and bilateral thalamus).

Conclusion. The interaction of C9orf72 and GRN gene defects results in mixed behavioral and language impairments. The pattern of grey matter atrophy recapitulates MRI alterations typical of the two single gene mutations. Rare cases of patients carrying double mutations would help disentangling the role of each gene in determining dementia.

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240. Double-blind randomised study to assess IFB-088 plus riluzole vs placebo plus riluzole in patients with bulbar-onset amyotrophic lateral sclerosis

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Background

Endoplasmic reticulum (ER) stress and activation of unfolded protein response (UPR) signaling pathways have been shown to be involved in the pathophysiology of amyotrophic lateral sclerosis (ALS). Targeting components of the UPR pathway and consequently modulating the proteostatic capacity of motor neurons is therefore a potential therapeutic target. Guanabenz that modulates the phosphatase complex protein phosphatase 1 regulatory subunit 15A / protein phosphatase 1c (PPP1R15A/PP1c) showed statistically significant benefits in a randomized double-blind phase 2 study, especially in patients with bulbar-onset ALS; however unintended hypotensive effects jeopardize further developments. IFB-088 (icerguastat) is a close analogue of guanabenz with similar cytoprotective effects but devoid of hypotensive activity. It has shown to be efficacious to reduce protein burden and its consequences in in vitro and in animal ALS models. A favorable safety has been demonstrated in a phase 1 study in healthy volunteers.

Objective

The primary objective of the study is to assess the safety of IFB-088 50 mg/day, in patients with bulbar-onset ALS. Secondary objectives include evaluation of efficacy, pharmacokinetics, quality of life and biomarkers.

Study design

Prospective, international, randomised, double-blind, placebo controlled, multicentre, parallel group phase II study. Patients are randomised 2:1 to receive IFB-088 50 mg/day + riluzole 100 mg/day or placebo + riluzole 100 mg/day and treated for a period of 6 months. 50 patients should be included in total. Eligible subjects are adult patients with probable or definite ALS with bulbar onset of disease, onset of symptoms ≤ 18 months prior to screening, SVC > 60% of predicted value for age and sex, ALSFRS-R score ≥ 36, treatment with riluzole 100 mg/day, at stable dose since at least one month. Patients with non-progressive or very rapidly progressing ALS, known other significant neurological disease, non-invasive ventilation, tracheotomy, weight loss ≥ 10% or BMI <18 kg/m2 at screening, dementia or other severe active psychiatric illness, significant pulmonary disorder not attributed to ALS are excluded. Efficacy will be assessed using ALSFRS-R, ALS-MITOS and King's College scores, respiratory function, and bioimpedance analysis. Quality of life will be evaluated using the ALSAQ-40 questionnaire. Main biomarkers include neurofilament light and heavy chain, TDP-43, neuroinflammatory and oxidative stress markers

241. Effect of edaravone therapy in Turkish amyotrophic lateral sclerosis (ALS) patients

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Objective: Oxidative stress caused by free radicals has been implicates in the pathogenesis of amyotrophic lateral sclerosis (ALS). Edaravone (also known as MCI-186) a free radical scavenger was approved as an ALS treatment in 2015 in Japan. This study aim to investigate of edaravone on ALS patients in the Turkish population

Material-Method: This study included 50 ALS patient were treated edaravone . All patients were diagnosed with ALS according the revised El Escorial criteria. In this study, clinical parameter including age, sex, age of onset, ALSFRS score evaluation, duration of diseas, respiratory function (FVC), mortality rates and nutritioanal function. Edaravone was administered twice a day 30 mg / 20 ml, IU for five days on one ciycle, two ciycle in one month, for 6 months. Some of patients used for a long time (at last 2 years). We don't see any side effect of edaravone.

Conclusion: This study was single anter, open label study on use of edaravone in ALS patients. This showed that edaravone which showed slow progression and decreased mortality of our patients.

242. Effect of tauroursodehoxycholic acid on survival and safety in amyotrophic lateral sclerosis: a retrospective population-based cohort study

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Importance: Oral tauroursodeoxycholic acid (TUDCA) is a drug currently tested in Amyotrophic Lateral Sclerosis (ALS). While results of phase 3 clinical trials on this treatment are awaited, TUDCA is easily administered and reachable for ALS patients.

Objective: To evaluate overall survival and safety profile in patients with ALS from Emilia Romagna Region (Italy) treated with TUDCA in a real-world setting.

Design: Multicenter, propensity score–matched observational cohort study. Propensity score matching was based on age at onset, sex, phenotype, diagnostic latency, ALSFRS-R at first visit, disease progression rate at first visit, BMI at diagnosis.

Setting: The study was conducted between January 2015 and February 2022 in Emilia Romagna Region where a population registry collects key clinical features from all specialized MND centers in the region since 2009, and where TUDCA has been prescribed, after approval from the regional rare diseases technical group, since 2015 by the same centers.

Participants: Of the 627 patients screened, 86 patients with ALS treated with TUDCA for at least three consecutive months were matched using propensity score analysis with 172 ALS patients receiving usual care. All patients were residents of Emilia Romagna Region and regularly followed till 1st February 2022.

Exposure: Oral TUDCA administration versus standard therapy.

Main Outcomes and Measures: primary study outcome was survival difference between TUDCA exposed and unexposed patients. Secondary outcomes were the rate of decline of ALSFRS-R from onset to last visit and frequency and time to supporting procedures.

Results: 86 patients assumed TUDCA; 64 were male (74.4%) and mean age was 58.2 years(SD 9.2). Median overall survival time was 49.6 months among TUDCA treated patients (95%CI, 41.7-93.5) and 36.2 months in controls (95%CI, 32.7-41.6), with a lower risk of death for higher dosage TUDCA-exposed patients (HR 0.56; 95% CI, 0.38–0.83; P=0.004).

No differences were observed in terms of rate of ALSFRS-r decline or time to support procedures. TUDCA was well tolerated except for a minority of patients (n=7, 8.1%) who presented excessive diarrhea, gastrointestinal discomfort or skin reactions and decided to discontinue the drug.

Conclusions and relevance: This study showed that ALS patients treated with TUDCA may have survival benefit. Additional prospective studies are needed to confirm its efficacy and safety in MND clinics.

243. Enlarged perivascular spaces in the midbrain are more prevalent in ALS and are associated with primary motor cortex atrophy

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Background: Amyotrophic Lateral Sclerosis (ALS) is a fatal disease causing motor neuron degeneration with large variability in age of onset, symptomatology and survival. While the cause of ALS remains elusive, recent findings implicate vascular remodeling with altered perivascular fibroblast activity preceding disease onset and ex vivo findings of enlarged perivascular spaces (EPVS). Perivascular spaces are also a cornerstone in the glymphatic system, which highlights the potential role of EPVS as an in vivo imaging biomarker in ALS.

Aim: To topographically investigate the prevalence of EPVS in ALS and their clinical correlates.

Methods: This prospective study consecutively recruited persons with ALS from the Department of Neurology at Karolinska University Hospital and neurologically healthy controls. Brain MRI was performed on a Siemens Prisma 3 Tesla scanner. FreeSurfer was used for brain volumetry and a 3D computational framework with a morphological-based filter (RORPO) was used to segment EPVS in regions of interest (centrum semiovale, basal ganglia and midbrain). Motor function was assessed using the revised ALS functional rating scale (ALSFRS-R) and cognitive function using the Edinburgh cognitive and behavioral ALS screen (ECAS). Linear regression analyses and student's t-test were performed in SPSS. The study was approved by the Swedish Ethical Review Authority and informed consent was obtained from all participants.

Results: In total, 88 ALS patients (63.8±11.7 years, 52 males) and 17 controls (58±14.0 years, 7 males) were included. The EPVS count, adjusted for age and sex, in the midbrain was significantly higher in ALS (N 17±10 vs. 10±7.9, P=0.009) and tended to be higher with shorter disease duration (std. β=-0.21, P=0.067), but was not associated with disease progression (P=0.25). ALS patients had a thinner motor cortex (2.36±0.11 vs. 2.44±0.09 mm, P=0.004), which was associated with higher midbrain EPVS count (std. β=-0.27, P=0.015). There was a lack of associations between EPVS count and clinical/paraclinical data such as progression rate, ALSFRS-R scores and ECAS scores.

Conclusions: Enlarged perivascular spaces in the midbrain are more common in ALS and are associated with motor cortex thinning. Our findings highlight the possible importance of EPVS as an in vivo biomarker and that vascular remodeling may contribute to ALS pathophysiology. Further studies are needed to explore the diagnostic and prognostic value of EPVS in ALS.

244. Epidemiological and Phenotypic features in the French ALS population: A prospective study on a cohort of 1000 index cases.

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1: CRMR SLA, CHU Tours, 2: CCMR SLA CHU Angers, 3: CCMR CHU Bordeaux, 4: CCMR CHU Caen, 5: CCMR CHU Clermont-Ferrand, 6: CCMR CHU Dijon, 7: CRMR CHU Lille, 8: CRMR CHU Limoges, 9: CCMR CHU Lyon, 10: CRMR Marseille, 11 : CRMR CHU Montpellier, 12: CCMR CHU Nancy, 13: CRMR CHU Nice, 14: CRMR CHU Paris, 15: CCMR CHU Saint-Etienne,16: CCMR CHU Strasbourg, 17: CCMR CHU Toulouse, 18: CCMR CHU La Réunion, 19:CCMR CHU Rennes, 20:CCMR Brest, 21: Laboratory of Molecular Biology, CHU Nimes, 22: Laboratory of Molecular Biology CHU Paris, 23: Laboratory of Molecular Biology CHU Tours.

Amyotrophic Lateral Sclerosis is the most frequent motor neuron disease in adults which remains currently uncured. Many hypotheses have been addressed to explain decades of failures in clinical trials. One of the explanations rely on the wide heterogeneity of ALS.

In order to strikingly describe epidemiological characteristics and phenotypic features of ALS in France, we have conducted from 01/04/2021 to 30/03/2022 a prospective study on incident ALS cases. One thousand patients with ALS have been enrolled in the study at the time of the diagnosis and have been followed during one year.

The following parameters have been collected:Age of onset, Site of Onset, time from 1st symptoms to the diagnosis of ALS based on Airlie House criteria, the existence of familial neurological diseases. We also collected the weight at diagnosis and the weight loss from the first symptoms, the ALSFRS-r score at diagnosis and the slope of progression of the ALSFRS-r score, the KC and MiTos staging at diagnosis and the Forced Vital capacity. All of them also had a genetic analysis of the C9orf72 and SOD1 genes.

There were 905 sporadic ALS and 81 familiar forms. Among Sporadic ALS there were 427 definite ALS, 272 probable ALS and 200 laboratory-supported ALS and 40 definite, 25 probable and 15 laboratory-supported among familiar ALS. Mean age of onset was 63.7 years in FALS group and 67.3 in sporadic ALS.

The results of our cohort will be present more accurately during this meeting.

245. Epidemiology and Patient characteristics of Amyotrophic Lateral Sclerosis (ALS) in France using the SNDS database

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder and is the most common type of motor neuron disease (MND) in adults, accounting for ~90% of cases. The aim of this study was to provide estimates of descriptive epidemiology of ALS in France and to describe patient characteristics for the incident population. This study was conducted using the French national claims database (SNDS). We identified patients with ALS from 2012 to 2019 who had at least 2 events (or 1 event in the case of death) within 6 months: reimbursement of riluzole, and/or diagnosis of MND (ICD10 code G12.2 recorded during a hospitalization or registration in the long-term illness list). We excluded patients who had at least one diagnosis for selected neurological conditions 6 months after the first ALS event. We identified 18,289 incident cases, with a slight male predominance (56.06%) and an average age at diagnosis of 68.35 (standard deviation 12.50) years. Between 2012 and 2019, the crude prevalence of ALS increased steadily from 6.78 to 10.99 and from 9.24 to 14.08 per 100,000 for point and period estimates, respectively. The crude incidence ranged between 3.39 and 3.51 per 100,000. A slow increase in the mortality was recorded from 2.46 to 3.09 per 100,000, but the case fatality rate decreased from 26.61% to 21.96% during the same period. The period prevalence, incidence and mortality were higher in men over the entire period, but the case fatality rate was lower. The period prevalence and incidence were higher in the oldest age groups (60-70, 70–80 and 80+) but the disease was also present among younger groups. The period prevalence in the regions Provence-Alpes-Côte d'Azur, Occitanie, and Pays de la Loire was consistently among the highest in France. The period prevalence rapidly increased in La Reunion, rising from 5.15 to 18.66 per 100,000 between 2012 and 2019. While the incidence remained stable in France over time, there has been an increase in prevalence. Higher incidence rates were consistently observed in older patients, although heterogeneity in patient age was seen. Regional variations in prevalence were recorded and may be explained by the establishment of new ALS centers. Case fatality rate was higher in females indicating differing health outcomes by sex but total annual case fatality rate decreased during the study period. With over 1/5 patients with ALS dying each year, the results highlight the continued need for more efficacious treatments.

246. Epidemiology of Serum Neurofilaments in Amyotrophic Lateral Sclerosis – Population-based Evidence For Diagnostic and Prognostic Utility

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Background:

Neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) have been established as promising blood biomarkers in ALS. Measuring NfL and pNfH in an epidemiological registry could help to further characterize their diagnostic and prognostic utility.

Methods:

In the epidemiological ALS registry Swabia (Southern Germany), we measured NfL and pNfH in 1929 serum samples from 837 patients within the ALS spectrum and 577 sex- and age-matched controls using a latest generation automated immunoassay system. We performed analyses for the diagnostic and prognostic utility and quantified the impact of potential confounders.

Results:

At baseline, patients within the ALS spectrum (mean age 65 ±11 years) had a median disease duration of 12.1 months (IQR 7.1 – 19.3), a median ALSFRS-R sum score of 38 points (IQR 33 – 42), and a median disease progression rate (ΔFRS) of 0.75 pt/m. Demographic data did not significantly differ between patients and controls. Follow-up data for survival was available in all patients (70.2% had died at data cut-off, median survival time after disease onset 3.17 years). The prognostic utility was better for NfL than pNfH. Converting NfL baseline levels of all ALS patients into population-based Z-Scores enabled dividing the ALS population into eight prognostic survival subgroups based on a single NfL measurement: an increase of 0.5 on the Z-scale was associated with a decrease in mean survival time of 8.5 months. The diagnostic performance to separate ALS from controls was again better for NfL (ROC AUC 0.947 (95%CI 0.935 – 0.958)) than for pNfH (ROC AUC 0.867 (95%CI 0.848 – 0.886). We identified an age-dependent increase of NfL and pNfH levels, especially in controls, as the major confounder for NfL and pNfH levels at baseline and found that including age in diagnostic tests increases specificity and sensitivity.

Conclusion:

Our epidemiology of neurofilaments characterizes the biomarkers on a population level providing valuable information for numerous possible applications. We found that the diagnostic and prognostic utility of serum NfL is superior to serum pNfH in all domains and quantified the effects of age and BMI as confounders. Using population-based NfL Z-scores instead of raw NfL levels considerably improves the prognostic performance, which might be valuable information for using serum NfL as a surrogate marker in clinical trials and for counseling patients in everyday clinical practice.

247. Evaluating single and multiple ascending doses of WVE-004 in C9orf72-associated ALS and FTD: results from the FOCUS-C9 Trial

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WVE-004 is an investigational stereopure antisense oligonucleotide designed to selectively target pathological C9orf72 transcripts while sparing C9orf72 protein. FOCUS-C9 (NCT04931862) was a global Phase 1b/2a trial designed to assess the safety and tolerability, as well as the pharmacodynamic, pharmacokinetic, and clinical effects of single- and multiple-ascending intrathecal doses of WVE-004 in people with C9orf72-ALS and -FTD.

In the single-ascending dose portion of this study, patients were randomized to 4 dose cohorts (10 mg, n=2; 20 mg, n=8; 30 mg, n=5; 60 mg, n=3) and placebo (n=5). In the multidose portion of the study, 10 patients were continued from the single-dose period while 12 new patients were enrolled (n=22; 10 mg monthly, n=6; 10 mg quarterly, n=9; placebo, n=7). Three patients treated with placebo in the single-dose portion of the study continued with placebo in the multidose portion. WVE-004 was generally safe and well-tolerated across doses. Most adverse events (AEs) presented as mild in intensity and were related to disease progression or intrathecal administration.

In this planned analysis, we confirmed that WVE-004 leads to robust and sustained reductions in poly(GP) in CSF from baseline, with a maximal mean reduction of 51% in the 20 mg single dose cohort at Day 169 ($p=0.0006$), of 48% in the quarterly 10 mg dose ($p<0.0001$) at Day 113, and 50% ($p=0.0001$) in the monthly 10 mg dose at Day 169. In the 10 mg monthly cohort, mean decline in ALSFRS-R was statistically significantly greater than the placebo group at week 24 ($p=0.0009$), but these changes were not statistically different from a propensity score-matched natural history cohort from the PRO-ACT database. In the quarterly cohort, there was no difference in ALSFRS-R mean change between WVE-004 and placebo or the propensity score-matched natural history cohort at any timepoint through 24

248. Evaluating the Efficacy and Safety of Tofersen in Adults with ALS and a SOD1 Mutation: Results from the Phase 3 VALOR Trial and Open-Label Extension

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VALOR was a Phase 3 trial in which adults with SOD1-ALS were randomized 2:1 to receive tofersen 100 mg (3 doses given ~2 weeks apart, then 5 doses given every 4 weeks) or placebo intrathecally. The primary endpoint was change from baseline to Week 28 in the ALSFRS-R total score. Key secondary endpoints included change from baseline in total SOD1 cerebrospinal fluid (CSF) concentration, plasma neurofilament light chain (NfL) levels, percent predicted slow vital capacity (SVC), handheld dynamometry (HHD) megascore, ventilation-assistance free, and overall survival. Participants had the opportunity to continue in an open-label extension (OLE) upon VALOR completion. Data from VALOR and its OLE were integrated to evaluate the effects of early-start vs. delayed-start tofersen over time.

One hundred and eight total participants were enrolled (tofersen [n=72], placebo [n=36]). The results from the primary VALOR and integrated VALOR/OLE analyses have been previously reported. Statistical significance was not achieved on the primary endpoint of change in ALSFRS-R total score over 28 weeks in the enriched primary analysis population (n=60). However, consistent trends favoring tofersen at Week 28, and early-start tofersen at Week 52 and beyond were observed across measures of strength, function, quality-of-life (QoL), and survival. Serious neurologic events were observed in 6.7% of tofersen-treated participants.

Importantly, tofersen administration led to substantial reductions in plasma NfL, suggesting a slowing of axonal injury and neurodegeneration. The authors will present new modelling data demonstrating the correlation between these tofersen-driven reductions in neurofilament and clinical benefit (preserved function, strength, QoL, reduced risk of death-equivalent events) over time. Additional exploratory biomarker data will also be presented.

249. Evaluating the Safety, Tolerability, and Pharmacokinetics of QRL-101 in Two Phase 1 Studies: QRL-101-01 in Healthy Adults and QRL-101-02 in Adults With ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressively fatal, neurodegenerative disease resulting in the loss of motor neurons in the motor cortex, brainstem, and spinal cord.1 In ALS, evidence of increased cellular excitability in peripheral nerves and central motor neurons has been observed through advanced neurophysiological, imaging, pathological and biochemical techniques.2,3 Clinically, hyperexcitability has been correlated with decreased longevity in people living with ALS.4 QRL-101 is an investigational product targeting motor neuron hyperexcitability. Preclinical studies in models of ALS have indicated QRL-101, a potent, selective KCNQ2/3 channel positive allosteric modulator, may be effective in reducing motor system hyperexcitability in people living with ALS.

Methods: The safety, tolerability, and pharmacokinetics (PK) of QRL-101 will be evaluated in two, consecutive, randomized, placebo-controlled, double-blind, phase 1 studies. The first, QRL-101-01, is an ongoing first-in-human, single-ascending dose (SAD) study in approximately 72 healthy participants. The study design includes up to nine dose escalation cohorts of 8 participants each, randomized in a 6:2 ratio of study drug to placebo. Information from QRL-101-01 will be used to determine a safe and tolerable dose range for the subsequent multicenter multiple-ascending dose (MAD) study, QRL-101-02, which will evaluate QRL-101 in approximately 24 adults living with ALS. Both studies will utilize a sentinel dosing strategy, as well as multiple safety reviews.

Results: In both studies, the primary and secondary endpoints will be incidence of adverse events and measurements of the PK of QRL-101 at single or multiple doses, respectively. In QRL-101-02, additional exploratory endpoints will be evaluated to assess the impact of QRL-101 on disease state, quality of life, and electrophysiological markers of motor nerve excitability in people living with ALS.

Conclusions: The findings from these studies will be used to advance the development of QRL-101, and other next-generation precision medicines for people living with ALS and other neurodegenerative diseases.

250. Evidence for a Survival Benefit in ALS with CNM-Au8 Treatment: Updated Results from the RESCUE-ALS Trial Long-Term Open Label Extension

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Introduction

RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 in early sporadic ALS to evaluate the long-term safety and efficacy of CNM-Au8, with an ongoing open-label extension (OLE). CNM-Au8 is a suspension of clean-surfaced, catalytically active gold nanocrystals shown to enhance neuronal energy metabolism and reduce oxidative stress.

Methods

Study participants were randomized 1:1 to receive 30mg CNM-Au8 or placebo (p.o.) daily for 36-weeks during the double-blind portion of the study, followed by an OLE with CNM-Au8 (30mg/day). The trial enrolled 45 participants [n=23 active; n= 22 matched placebo)]. Thirty-six participants continued into the OLE: 20 of 21 eligible participants (95%) originally randomized to CNM-Au8; 16 of 19 eligible (84%) participants originally randomized to placebo. Since all current participants have been treated with long-term CNM-Au8 (median OLE exposure: 1.6 years, maximum OLE exposure: 2.3 years), survival analyses compared participants' observed survival to estimated median survival based on the validated ENCALS prediction model. Data were right-censored at either last study contact for any participants lost to follow-up, or as of the date of this submission (27-March-2023).

Results

Predicted median survival and 25-75% confidence intervals were derived from the published ENCALS model based on each participant's baseline characteristics. Kaplan-Meier analyses demonstrated a significant survival benefit associated with CNM-Au8 treatment (log-rank, $p<0.05$). CNM-Au8 treatment was well-tolerated and there were no significant safety findings reported during the OLE.

Conclusion

Results demonstrate improved survival with CNM-Au8 treatment compared to estimated median survival derived from the ENCALS prediction model.

251. Examination of Age, Period and Cohort Effects using a Partial Least Squares Regression (PLSR) model

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In the longitudinal analysis of disease trends, it is important to consider age, period and cohort effects which may have contributed to observed crude trends. In this analysis we have used a partial least squares regression model (PLSR) to investigate incidence and separate age, period, and cohort effects. We have examined the Irish population over the course of 25 years using data obtained from the Irish MND register.

Extracting two components the PLSR model explained 99.98% of variance and a jack-knife method was used to estimate 95% confidence intervals. There was evidence of linear and non-linear trends suggesting underlying epidemiological modifiers of disease presentation. An underlying complex age relationship has been demonstrated with both negative regression coefficients, for those aged between 35 and 60 (R2 = -5.54E-04), and positive relationships for those aged 65-79 (R2 = 7.46E-03). These findings support the multi-step hypothesis of ALS pathogenesis and an underlying relationship between environment and genetics.

There was also evidence of a significant cohort effect with elevations in the regression coefficients corresponding to the birth cohorts spanning 1927-1947; during the second world war and in the period following the Irish civil war. This is suggestive of significant epigenetic modification in the disease pattern within the Irish population as a result of whole population change (R2 = 9.35E-04).

A period effect was also demonstrated which likely corresponds to a change in case ascertainment over the course of the last 25 years, which may be due to a number of factors (R2 = 1.07E-02). This is the first demonstration of significant age and cohort effects within an Irish population reflecting important environmental conditions implicated in disease modification.

252. EXAMINING COGNITION AND BEHAVIOURAL PROFILE IN ALS-FTD SPECTRUM

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Background: Cognitive and behavioural clinical manifestations in the continuum of behavioural variant frontotemporal dementia (bvFTD) and amyotrophic lateral sclerosis (ALS) is incompletely characterized, mostly because only a reduced number of neuropsychological tests are performed in clinical practice. This prospective comparative study aims to determine whether there are phenotypic differences in cognition and behaviour in ALS and bvFTD patients.

Methods: Patients with ALS (n=11), bvFTD (n=11) and healthy controls (n=22) were recruited. Participants were administered the Edinburgh Cognitive and Behavioral ALS Screen (ECAS), which comprises tests of language, verbal fluency, executive functions, memory and visual-spatial functions. They also carried out analogous, full-length cognitive tests (ACE-III and Neuronorma battery). Neuropsychiatric symptoms (NPI and Lille's Apathy Rating Scale) and social cognition skills (Hayling test) were evaluated. Blood DNA samples were used for a genetic screen panel for known ALS and FTD mutations.

Results: Preliminary highly significant differences were elicited in attention ($p<0.001$), executive functions ($p<0.001$), language ($p<0.001$) and memory ($p<0.05$) reflecting poorer performance in bvFTD patients compared to ALS. No significant differences in verbal fluency was found between ALS and bvFTD but were found in bvFTD compared to controls ($p<0.05$). There were strong correlations between performance on ECAS subtests and analogous cognitive tasks. Social cognition and neuropsychiatric symptoms were reported more frequently in bvFTD than ALS ($p<0.05$). Behaviour in ALS was dominated by apathy ($p<0.05$).

Conclusions: These preliminary findings suggest differences in cognition and behaviour pattern between bvFTD and ALS. Overall, cognition is severely impaired in bvFTD compared to ALS. While changes in social behaviour are prominent in bvFTD, apathy was more frequent in ALS. Identification of a distinct neuropsychological phenotype in FTD and ALS may have a fundamental clinical role for early diagnosis, disease management and care planning and theoretical implications for our understanding of the relationship between ALS and bvFTD.

253. Exploring treatment burden and adherence to treatment in ALS patients: a prospective multicentric study

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Adherence to medication regimens can be challenging for patients with ALS, especially those who develop dysphagia. Medications may need to be crushed, mixed with a liquid, or delivered through a feeding tube. This can be challenging for patients and caregivers, as it may require additional time and effort to prepare medications and administer them. In addition to the physical burden of preparing and administering medications, changes in drug therapy can be dangerous for patients impacting medication efficacy and safety. The aim of the study was to investigate the role of treatment burden in improving treatment management in a cohort of incident ALS patients. ALS patients were prospectively recruited at 3 Italian reference centres for ALS. Demographic and clinical features were collected, as well as the level of treatment burden, functional impairment, the level of dysphagia, the treatment adherence's level, the somatic symptom burden, quality of life, comorbidities and ongoing medications. A total of 103 ALS patients were recruited. The 64% of ALS patients reported a none to low treatment burden, whereas the 36% resulted to had medium to high treatment burden level. The level of treatment burden was significantly related to the patient's need to change drugs' formulation (p=0.0469), regardless the level of somatic symptom burden, disease duration, functional impairment, dysphagia level, comorbidities and number of taken medications. Moreover, higher level of treatment burden was significantly related to a worse INQoL treatment perceived effect (p=0.0323) and to a worse INQoL total score (p=0.0024), independently from somatic symptom burden, disease duration, functional impairment, dysphagia level, comorbidities and number of taken medications. Finally, higher treatment burden's level was significantly related to a poor treatment adherence (p=0.0089), regardless the level of somatic symptom burden, disease duration, functional impairment, dysphagia level, comorbidities and number of medications. adherence to drug therapy is critical in the management of ALS. The need to change drugs formulation increases the treatment burden with a negative impact on patient's quality of life and adherence to treatment. Thus, the most adequate drugs formulation, such as oral solution when available, should be considered since the beginning of ALS symptoms and indipendently to dysphagia, in order to limit treatment burden and guarantee adherence to treatment.

254. Factors Impacting Trial Participation in People with Motor Neuron Disease

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Introduction: The Motor Neuron Disease Systematic Multi-Arm Randomised Adaptive Trial (MND-SMART) is a multi-site UK trial seeking effective disease modifying drugs. Historically, neurological trials have been plagued by suboptimal recruitment and attrition. Failure to recruit and retain participants may influence sample representativeness, result in premature termination, or invalid conclusions.

Aim: This study investigates person-specific factors affecting recruitment and retention of people with MND to MND-SMART. Improved understanding of these factors may improve trial protocol design, optimise recruitment and retention.

Methods: Participants were recruited through the Scottish MND register CARE-MND (Clinical Audit Research Evaluation) and completed a series of questionnaires to evaluate neuropsychiatric symptoms, quality of life and attitudes to trials. This was supplemented with data on clinical phenotype, cognition and physical functioning. Caregivers completed the brief apathy scale. 12 months on we used MND-SMART recruitment data to establish if members of our cohort engaged with the trial.

Results: 120 people with MND completed questionnaires for this study. Mean age at participation was 66y (SD = 9y), 14% (n = 17) were long survivors and 68% (n = 81) male. The majority, 60% (n = 73), had the ALS sub-type. 73% (n = 88) had a caregiver co-participating with them. Of the 120 study participants, 50% (n = 60) were randomised to MND-SMART and 78% (n = 94) expressed interest in participating by attending a screening appointment, completing online forms or contacting the trial team. After one year 65% (n = 39) of randomised participants remained in MND-SMART. One individual chose to withdraw and the remaining participants died. Only age was a significant predictor for trial participation, (OR = 0.92, 95% CI = 0.88 to 0.96, p = 0.000488), with the likelihood of participation decreasing as participant age increases.

Conclusions: The findings show that people with MND are highly motivated to engage in research, but older individuals remain significantly less likely to participate. For every increase in 1 year of age, the odds of participating in MND-SMART decreased by 1%. Further work to identify barriers to participation and retention must remain a priority. We recommend the inclusion of studies to explore characteristics of prospective and current participants alongside trials, either as sub-studies or additional analyses of trial data.

255. Familial ALS with a Novel ANXA 11- Mutation Presenting with a Proximal Pattern

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Introduction/Background: Variants in the annexin A11 gene have been reported to be associated with ALS, but very few familial cases have been identified so far.

We report a patient with a familial ALS with a heterozygous ANXA-11Mutation (NM_145868.1:c.1210C>T/p.(ARG404Trp)) presenting with a patchy phenotype and atypical features.

Case description: A 49-year-old male presented with a 18 month history of progressive proximal weakness of his left leg, not able to climb stairs or stand up from the floor unsupported.

On the first neurological examination we found a positive Trendelenburg sign, isolated muscular wasting of the glutei and the quadriceps muscles with exaggerated tendon reflexes on the left side. ENMG revealed chronic and active signs of denervation in all limbs, but not in the paraspinals.

Over the past 30 months the patient visited our ALS Clinic regularly. Proximal weakness in his legs progressed with periods of clinical stabilisation. In addition he developed wasting of his right shoulder starting rapidly which stabilized in the further disease course.

An early PEG and cystostomie were necessary to treat malnutrition due to poor appetite and overflow bladder. Currently the patient is still able to live independently and reports a good quality of life.

Family history revealed, that his father and a paternal uncle died early after a period of muscle wasting and weakness, initially presenting with a very similar gait disturbance at the age of 55 (father) and in the early 40s (uncle).

Through Whole Exome Capturing and Next Generation Sequencing a missense variant in the ANXA11 gene was found, which leads to the exchange of a highly conserved amino acid with an average physicochemical difference between arginine and tryptophan. NM-145868.1:c.1210C>T/p.(ARG404Trp). We searched this genomic variant in the ClinVar and HGMD- databases and found no prior description.

Conclusions: Although no genetic analysis was performed in the father and uncle, this case reports suggest that the reported gene mutation is disease-causing. This case report widens the spectrum of genotype- phenotype correlations in patients with ALS and ANXA 11 gene mutations.

256. First-in-human study of safety, tolerability, PK, and PD of SAR443820, a centrally penetrant RIPK1 inhibitor in healthy participants

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Introduction: Receptor-interacting serine/threonine protein kinase 1 (RIPK1) regulates inflammatory signalling and necroptotic cell death and is implicated in neurodegenerative pathology. SAR443820 (DNL788), a selective, oral, centrally penetrant RIPK1 inhibitor, is a promising therapeutic option in amyotrophic lateral sclerosis (ALS). We assessed safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of SAR443820 in first-in-human (FIH), phase 1, randomized, double-blind, placebo-controlled, single ascending dose (SAD; Part 1a) and multiple ascending dose (MAD; Part 2) study in healthy participants. Part 1b was a separate open-label single dose study for assessing SAR443820 levels in cerebrospinal fluid (CSF) to confirm CNS-penetrance.

Methods: In Part 1a, 4 cohorts (n=8 each; 6 SAR443820, 2 placebo) received SAD of SAR443820 (up to 4-fold the lowest dose) or placebo in fasted conditions. Part 1b included 2 single dose cohorts (n=6 each) receiving the lowest and 4-fold the lowest doses of SAR443820 in fed conditions. In Part 2, 4 cohorts (n=10 each; 8 SAR443820, 2 placebo) received 14 days of SAR443820 or placebo in MAD as once/twice-daily dosing regimens in fasted conditions.

Results: SAR443820 was well-tolerated in all studies, with no treatment-related serious adverse events (AEs) or AE-related permanent treatment discontinuation. Most common AEs were dizziness and headache. No clinically meaningful changes were noted in haematology, chemistry, vital signs, or electrocardiogram parameters. Overall, no major deviations were seen from dose proportionality for maximum concentration (Cmax) and area under the curve (AUCs) over the range of SAR443820 doses. Mean plasma half-life (t1/2z) ranged from 6-8h and 7-9h, following single and repeated SAR443820 doses, respectively. Mean CSF-to-unbound plasma concentration ratio indicated optimal CNS-penetrance. Maximum median inhibition of phosphorylated-Ser166-RIPK1 levels across all SAR443820 groups in SAD and MAD studies reflected a marked target engagement.

Conclusions: FIH study demonstrated that single and repeated SAR443820 doses were generally safe and well-tolerated, with favourable PK, high CNS-penetrance, and robust RIPK1-target engagement. These results support further development of SAR443820 in an actively recruiting phase 2 Himalaya trial (NCT05237284) in patients with ALS.

257. Gastrostomy tube – is it for me?' A web-based decision aid to support people with motor neurone disease considering a gastrostomy tube

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Introduction

When eating and drinking become difficult, people living with motor neurone disease (plwMND) may be offered a gastrostomy tube (GT) to administer food, fluids and medication. However, high quality evidence that GT improves either nutritional, survival or quality of life outcomes is lacking. Patient decision aids (PDA) support shared decision making by providing evidence-based information, communicating the risks and benefits associated with each option, checking understanding and clarifying personal values and preferences.

Methods

The three-phased DiAMoND study aimed to co-produce and pilot test a web-based PDA for plwMND considering having a GT placed. Participants were plwMND, carers and healthcare professionals (HCPs). In Phase 1, PDA content and design were informed by semi-structured interviews, two literature reviews, a prioritisation survey and international PDA standards. In Phase 2, a prototype PDA was tested with users and improved iteratively using feedback from surveys and 'think-aloud' interviews. In Phase 3, the PDA was used by patients who then completed validated questionnaires. HCPs provided feedback in three focus groups.

Results

A total of 16 plwMND, 16 carers and 25 HCPs took part in Phases 1 and 2. From the interviews and literature reviews, 82 items of content were generated, and 63 were retained following the prioritisation survey with. Following the iterative improvement process in Phase 2, 17 plwMND completed questionnaires after using the PDA in Phase 3. Most plwMND (94%) found the PDA completely acceptable and would recommend it to others in their position, had no decisional conflict (88%), and were well prepared (82%) and satisfied with their decision making (100%). The 3 groups were attended by a total of 17 HCPs. Feedback was generally positive and implementation strategies were discussed.

Conclusion

Gastrostomy Tube: Is it for me? is a valuable tool to support plwMND and HCPs through the GT shared decision making process. It was co-produced with stakeholders and found to be acceptable, practical and useful to plwMND. The PDA is hosted by the MND Association on its website and is freely available.

258. Genetic characterization of primary lateral sclerosis

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Background and objectives: Primary lateral sclerosis (PLS) is a motor neuron disease characterised by loss of the upper motor neurons. Most patients present with slowly progressive spasticity of the legs, which may also spread to the arms or bulbar regions. It is challenging to distinguish between PLS, early-stage amyotrophic lateral sclerosis (ALS) and hereditary spastic paraplegia (HSP). The current diagnostic criteria advise against extensive genetic testing. This recommendation is however based on limited data.

Methods: We aim to genetically characterize a PLS cohort using whole exome sequencing (WES) for movement disorders (338 genes) and ALS (26 genes) and C9orf72 repeat expansions. Patients fulfilling the definite PLS criteria by Turner et al. and with available DNA samples of sufficient quality were recruited from an on-going, population-based epidemiological study. Genetic variants were classified according to the ACMG criteria and assigned to groups based on disease association.

Results: WES was performed on DNA samples from 139 patients and the presence of repeat expansions in C9orf72 was analysed separately in 129 patients. This resulted in 31 variants of which 11 were (likely) pathogenic. (Likely) pathogenic variants resulted in 3 groups based on disease association: ALS-FTD (C9orf72, TBK1), pure HSP (SPAST, SPG7), ALS-HSP-CMT overlap (FIG4, NEFL, SPG11).

Discussion: In a cohort of 139 PLS patients genetic analyses resulted in 31 variants (22%) of which 11 (8%) (likely) pathogenic associated with different diseases (predominantly ALS and HSP). Based on these results and the literature we advise to consider genetic analyses in the diagnostic work-up for PLS.

259. Global Fundamental Rights in ALS/MND Survey: A look into the global results.

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1 The International Alliance of ALS/MND Associations.

The Alliance has developed a guiding document on fundamental rights for people living with ALS/MND and caregivers of people living with ALS/MND, that states the aspirational rights of the global community. These rights are reviewed annually and are the basis for the survey we ran in 2021.

Access to and respect of these Fundamental Rights is inconsistent around the world and is dependent on multifactorial inputs such as economics, healthcare systems and professionals, and culture which leads to inconsistent quality of life for people living with ALS/MND.

The survey addressed a range of important topics in the ALS/MND community which included an assessment of how PALS rights are respected globally, access to healthcare, treatments, health professionals, services, support and much more.

We would like to share our results on the following areas:

o Respect for the global fundamental rights.
o How access to highest quality of care is globally unbalanced between the Global North and the Global South.
o Access is the biggest issue for treatments (Clinical Trials, Approved Drugs), Assistive Devices and Genetic counselling. And how it is unbalanced between the Global North and the Global South.
o The right to information and education is better respected in countries where English is spoken.
o Caregivers feel unsupported with little access to counselling, and emotional support programs.

We would welcome the opportunity to share our findings.

260. HTT and ATXN2 repeat expansions increase risk of ALS in a Norwegian ALS cohort: The GAIN study

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Background: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease with genetic, metabolic and environmental risk factors. Genetic repeat expansions are a frequent cause of neuronal degeneration both in ALS and in related neurodegenerative diseases such as ataxias, frontotemporal dementia, Huntington's disease and Kennedy's disease. Further, it appears to be a genotype-phenotype correlation, where repeat expansions in the same gene may result in multiple neurodegenerative phenotypes. Here, we investigate repeat expansions in AR, ATXN1, ATXN2 and HTT in a Norwegian ALS cohort.

Methods: Norwegian ALS patients (n=414) and motor neuron healthy controls (n=1092) were analyzed for repeat expansions in AR, ATXN1, ATXN2 and HTT by next-generation sequencing and ExpansionHunter. Identified repeat expansions were validated by traditional fragment analysis.

Results: Repeat expansions in HTT and ATXN2 were associated with increased risk of ALS. Six ALS patients (1.45%) and three controls (0.28%) carried repeat expansions (36-40 repeats) in HTT (OR = 8.74, p = 0.004), whereas seven ALS patients (1.69%) and four controls (0.37%) carried repeat expansions (29-34 repeats) in ATXN2 (OR = 6.09, p = 0.007). ALS diagnoses were re-evaluated and confirmed by two independent neurologists. In addition, one ALS patient carried a pathogenic repeat expansion (45 repeats) in AR. Repeat expansions in ATXN1 (≥34 repeats) were not associated with increased ALS risk (p>0.05) in this cohort.

Conclusion: Having more than 36 repeat expansions in HTT and more than 29 repeat expansions in ATXN2, increases the likelihood of developing ALS.

261. Hypothesis-free Mendelian Randomisation Identifies New Metabolites Linked to Risk of ALS

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ALS, a rapidly developing and invariably fatal neurodegenerative disorder, is characterised by the progressive loss of motor neurons. ALS is an archetypal complex disease, with 10% of patients suffering from a monogenic disease; the vast majority of cases are sporadic, caused by a combination of genetic and environmental factors. With two-sample Mendelian randomisation (MR), causal inference can be made between various exposures and disease risks, such as serum concentrations of the entire set of metabolites. Summary statistics from genome-wide association studies (GWAS) of 575 metabolites were compared with those from a GWAS of amyotrophic lateral sclerosis (ALS) consisting of 29,612 ALS patients and 122,656 controls. Unbiased MR using the inverse variance weighted (IVW) estimate and weighted median causally associated five metabolites with risk for ALS after stringent Bonferroni multiple testing correction. Two hits are in the carnitine synthesis pathway – carnitine has been previously linked to the severity of ALS via a role in energy metabolism within motor neurons; this is the subject of an ongoing clinical trial (ALSUntangled). The remaining hits are being investigated with a combination of protein, metabolite and rare variant analyses. Follow-up studies will include evaluation within in vitro and in vivo models of ALS.

262. Impact of bulbar involvement on respiratory impairment in ALS patients

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Introduction:

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that leads to progressive muscle weakness and atrophy. Respiratory failure is the most common cause of death in ALS, although bulbar involvement also impacts on quality of life and survival. However, the relationship between bulbar involvement and respiratory function is still unclear. This is partly due to methodological biases in some respiratory function tests, which may be affected by orolingual weakness. This study investigates whether bulbar involvement is associated with greater respiratory impairment in ALS patients.

Methods:

Retrospective analysis of prospectively collected data from the registry of ALS patients visited between 2016 and 2022 in our center, in whom a respiratory assessment was performed in parallel with an examination of maximum tongue pressure (MTP), as a measure of bulbar weakness. Respiratory tests included those using a mouthpiece, and therefore could be affected by orolingual weakness: forced vital capacity (FVC), slow vital capacity (SVC), peak cough flow (PCF), maximal inspiratory (MIP) and expiratory pressures (MEP). Additionally, sniff nasal inspiratory pressure (SNIP) was performed, for whose performance is independent of the bulbar impairment since a mouthpiece is not needed. Demographic and functional data (ALSFRS-R) were also analyzed.

Results:

181 respiratory and MTP assessments of 64 ALS patients were evaluated. MTP was correlated with respiratory outcomes measured with a mouthpiece; SVC %pred (R:0.311; p:0.003), FVC %pred (R:0.404, p<0.001), PIM cmH2O(R:0.411, p<0.001), PEM cmH2O (R:0.366, p<0.001). In addition, it was also correlated with SNIP cmH2O (R:0.372, p<0.001). The correlation between MTP and SNIP was maintained even adjusting with the time of duration of the disease (R:0.235, p=0.004) and site of onset (R:0.221, p=0.007). Also, ALS patients with a respiratory impairment assessed with SNIP (p=0.004) presented lower MTP (median 26kPa, IQR 17-42) than those without respiratory affection (median 42kPa, IQR 24-50).

Conclusions:

We found that lower MTP is associated with worse respiratory function, regardless of the respiratory assessment method used. These findings highlight the importance of assessing bulbar involvement in ALS patients to identify those at higher risk of respiratory failure.

263. Impact of COVID-19 pandemic on the progression and survival of patients with amyotrophic lateral sclerosis

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Background: Some studies have analysed the effect of the COVID-19 pandemic on the quality of life of amyotrophic lateral sclerosis (ALS) patients, but little is known about its impact on the progression and survival of ALS patients. The purpose of this study is to describe the risk of SARS-COV2 infection, hospitalisation and death among ALS patients, and to compare the survival of this population during the pandemic with an equivalent pre-pandemic period.

Methods: In this retrospective descriptive study three cohorts of ALS patients were analysed: Pandemic cohort: patients visited between March 15th 2020-July 15th 2022; survival pre-pandemic cohort: patients who died between March 15th 2017-March 14th 2020; survival post-pandemic cohort: patients who died between March 15th 2020-March 14th 2022. Demographical and clinical data were collected and statistical analysis were performed using R and RStudio.

Results: Among 263 ALS patients in the pandemic cohort, the infection rate was 14.34 per 100 person-years. Most infections (68%) occurred during the sixth wave (Nov 2021–Jan 2022) and most patients (67%) were vaccinated at the time of infection. The hospitalisation rate due to COVID-19 was 4.16 per 100 person-years. Hospitalised patients had longer disease duration (35 vs 26 months) and were more frequently women (61%) and NIV users at the time of infection (56%). The multivariable model confirmed NIV use prior to infection as a risk factor for hospitalization (OR=2.075, p=0.003) independent of age, sex and vaccination status. Within 30 days after infection, 7% of non-ventilated patients started NIV (3% among non-hospitalized vs 22% among hospitalized patients), 8% of patients died, all of whom had been hospitalized (28% of hospitalized patients). Both pre and post-pandemic cohorts showed similar baseline demographic and clinical characteristics. There was no difference in the median survival of patients who died before and during the pandemic. In the Cox model, after adjusting for other co-variables, no statistically significant effect of the pandemic was found in the survival of ALS patients (HR=1.02, p=0.89).

Conclusions: This study suggests a higher risk of severe COVID-19 among ALS patients requiring NIV. Nevertheless, the overall impact of the pandemic in morbidity/mortality of ALS patients was mild, probably due to a relatively low infection rate, a high vaccination rate and an adequate access to healthcare resources among patients during the pandemic.

264. Impairment of oculomotor functions in patients with early to advanced ALS

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Background: Advanced ALS patients in an incomplete locked-in stage (iLIS) are tetraplegic and anarthric, but retain control of their eye movements. Therefore, iLIS patients rely on eye tracking computer systems (ETCS) for independent, complex communication. However, research revealed that oculomotor function is affected in ALS, mainly by pathological processes in non-motor brain regions. Oculomotor deficits can be assessed with ETCS and help to detect cognitive function decline. Nevertheless, studies on oculomotor function in ALS are rare and focus on earlier disease stages.

Methods: We developed an eye tracking test battery of oculomotor functions and assessed (i) ALS patients in an iLIS (n=22), (ii) ALS patients in early to mid-disease stages (n=44), and (iii) healthy controls (n=32). Groups were compared by means of marginal models using generalized estimating equations.

Results: We found significant deteriorations of ALS patients' performance in all oculomotor tasks, which were strongest for iLIS patients. Compared to healthy controls, patients showed longer latencies of reflexive and voluntary saccades and the reflexive saccades were hypometric. Patients made more errors in the antisaccade task, indicating deficits in inhibitory control. Smooth pursuit was slower in both patient groups and in iLIS patients more frequently interrupted by catch-up saccades. Moreover, early- to mid-stage ALS patients with bulbar compared to spinal onset showed greater smooth pursuit impairments and higher antisaccade error rates.

Discussion: The results reveal a pronounced decline of reflexive and voluntary oculomotor function during the course of ALS up to iLIS. This progression indicates pathological changes in the underlying neuronal networks, reaching from brainstem over midbrain to specific (pre)frontal areas. The findings highlight that oculomotor parameters can serve as bio- and progression markers in ALS and especially in iLIS, when other motor functions are lost. They may also provide insight into executive dysfunctions that are otherwise difficult to detect in iLIS. Moreover, deficits may jeopardize the use of ETCS for communication and thus self-determination and quality of life. In our subsequent project ADAPTIV, we investigate the natural course of oculomotor, cognitive and language functions in ALS by means of a comprehensive, gaze controlled test battery. The study focuses on biomarkers and ways to adapt ETCS to deteriorations of the examined functions.

265. Interdisciplinary approach in Amyotrophic Lateral Sclerosis: the case of the ALS Centre of Uruguay, the CELAU Program

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The beneficial effects of a multi-disciplinary approach in ALS are well established. Uruguay is a South American country with a population of about 3.3 million, with more than half residing in Montevideo, the capital city. Data collected from a two-year period (2002-2003) showed a similar incidence and prevalence of ALS compared to global numbers, but with a nine-month shorter survival time than a similar French population. This was attributed to limited access to riluzole, heterogeneity of medical care, and the lack of a referral ALS Centre.

Our group, originated from the collaboration aimed to generate translational ALS research, between the Institute of Neurology (IN) of the University Hospital, called Hospital de Clínicas (HC), and the Laboratory of Cellular and Molecular Neurobiology (NBCM) of the Histology Department of the Faculty of Medicin. In 2014, Tenemos ELA, an organization of patients, relatives, and friends, approached the IN to discuss the need for an interdisciplinary approach, leading to the integration of other faculty departments and the formation of the ALS Centre of Uruguay (Centro de ELA del Uruguay - CELAU). CELAU's purpose is to promote an interdisciplinary and integral approach to ALS through assistance, teaching, research, and activities for the ALS community. CELAU is composed of basic and clinical academics, neurologists, rehabilitation and physical medicine specialists, psychologists, nutritionists, speech therapists, and Tenemos ELA volunteers. Its major activity is the ALS interdisciplinary Clinic at the HC, which operates once a month, but due to growing demand, consultations have been increased to weekly frequency from February 2023. Other important activities include workshops for patients, family and caregivers, update meetings for healthcare teams, and academic meetings.

In contrast with almost 100% of patients being initially from HC or other public Health Services, by 2021-2022, 32% of patients came from private Health Services and 75% were from Montevideo.

We also consider important to highlight that the CELAU Program is located at the HC, where nearly half of the undergraduate and most postgraduate medical students in the country are trained.

In conclusion, although the management is challenging, an interdisciplinary team for ALS is possible in Uruguay. We trust that the CELAU Program will generate benefits, both for the academic and healthcare staff, as well as for the patients.

266. Italian version of the Rasch-Built Overall Amyotrophic Lateral Sclerosis Disability Scale (ROADS): validation and longitudinal performance

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Objective: To validate an Italian version of the Rasch-Built Overall ALS Disability Scale (ROADS) in a broad population of patients and assess its longitudinal performance over time.

Methods: 270 ALS patients referring to the Motor Neuron Disease Clinic of the University of Padova and Modena (Italy) accepted to compile the Italian version of the ROADS and results were correlated with the ALSFRSr and ALSAQ-40 scores, FVC values, and creatinine or albumin blood levels. To verify test-retest reliability, patients were asked to fill in a second copy of the scale within 5-7 days. Thirty-nine patients compiled a further copy of questionnaire during the follow up visit (after 133 days on average) which allowed us a longitudinal assessment of the scale.

Results: We found a good external construct validity between ROADS and either ALSFRS-R (correlation coefficient = 0.85) or ALSAQ-40 (correlation coefficient = - 0.84). Test-retest reliability was excellent with a concordance-correlation coefficient of 0.93. Yet, we observed a significant correlation between changes over time of the ROADS normalised sum score (- 2.18 point loss per month) and those of both the ALSFRS-R (positive correlation; Rho = 0.64, $p \leq 0.0001$) or the ALSAQ-40 (negative correlation; Rho = - 0.60, $p = 0.014$).

Conclusions: The Italian version of ROADS proved to be a reliable marker to monitor overall disability in ALS patients. Further studies are necessary to assess its longitudinal performance.

267. Lateral hypothalamus-dependent sleep impairment in ALS

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ALS is a progressive motor neuron disease inexorably leading to a premature death. Sleep disturbances have been ascribed to respiratory insufficiency, muscle cramps, spasticity, or restless legs syndrome, all leading to increased wakefulness. However, a recent neuropathological study in ALS patients described a loss of orexin-producing neurons, a neuropeptide involved in sleep and metabolic regulation, undermining the idea that sleep alterations are linked to central and peripheral changes.

Sleep changes are poorly characterized in ALS, and their relationships to motor symptom onset, disease progression and orexin neurons remain unknown. Here, we used electroencephalography coupled with indirect calorimetry recordings to characterize sleep and energy metabolism in two mouse models of ALS -Superoxide Dismutase 1 G86R (Sod1G86R) and Fused in Sarcoma (FusΔNLS).

In both Sod1G86R and FusΔNLS mice, electroencephalograms showed an increase in wakefulness and a decrease in rapid eye movement (REM) episodes before the onset of major motor troubles. We did not observe an altered number of Orexin-positive neurons in the lateral hypothalamus of these mice. Moreover, Suvorexant®, a drug antagonizing both orexin receptors, induced an increase in REM sleep and a decrease in wake quantities compared to control in both mouse lines. Interestingly, Sod1G86R and FusΔNLS mice displayed an increase in body temperature, energy expenditure and locomotor activity, as well as a lower respiratory quotient that were successfully rescued in both mouse models by the drug.

Sleep analysis in presymptomatic gene carriers and ALS patients matched with healthy controls is ongoing.

Thus, our results show that two mouse models of ALS display sleep and metabolic impairments and provide pharmacological evidence for the involvement of orexinergic neurons in these defects.

268. Leading subdomains of the ALSFRS-R in the D50 progression model

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Background: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder with a heterogeneous clinical presentation. The predominant endpoint in therapeutic trials are based on the ALSFRS-R total score but not on the progression of dominant subscores such as the bulbar, gross motor or fine motor domain in the respective phenotype.

Objectives: Analyzing aggressiveness of different ALS onset types in ALSFRS-R subdomains

Methods: Subgroup analysis of ALS was determined by the revised El Escorial criteria based on the data collection from the Department of Neurology, University Hospital Jena between August 2007 and May 2021. After having given written informed consent, 547 patients with a ALSFRS-R > 1 were included with a mean of 7.4 scores/person (min 2; max 43). For comparison between different onset types we used relative disease (rD50). In this way the individual time was standardized and normalized. SPSS (Ver. 28.0; IBM Corporation, Armonk, NY, USA) was used for all statistical analysis. The visualization of data analysis was accomplished by Microsoft Excel.

Results: 33.5% of the included patients had a bulbar onset followed by cervical (30.9 %), lumbar (28.3%), spinal (4.9%) and general (0.5%). In rD50, the main subscore that correlated to the onset type of ALS had the main impact on the decrease of ALSFRS-R. Moreover, the progress of fine and gross motor function seemed to be concurrent. However, the breathing subscore (items 10-12) had only a low and late impact on the total score. Those results have been observed for all subscores except the axial onset type. In that type of ALS it was not possible to distinguish which subgroup is the main driver of the ALSFRS-R score.

Conclusion: The data analysis shows that a focussed mapping of the leading subscores suffice to map the whole disease course. That should be considered in future trials. The breathingl subscore seems to have a low impact on the ALSFRS-R drive while in this subtype a more detailed analysis is needed. Long-term survivors can confound the results. These results need to be further validated in multicenter cohorts.

269. Light The Way: Development of an Online Support Platform for People at Risk of Genetic ALS/FTD in the UK and US

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Objective: People at risk of genetic ALS/FTD (e.g. children of an affected patient) face a variety of challenges; those who learn they carry an ALS-causing gene may experience significant stress, panic, worry about developing ALS, and even suicidal ideation. However, there is surprisingly little formal support available. While experts suggest pre- and post-test genetic counseling are important, in practice this can be applied inconsistently and there are calls for the development of new guidelines to fill the gaps. Globally, there are insufficient genetic counselors to provide timely and adequate support for individuals, necessitating novel approaches.

Methods: With the support of scientific advisors and people at risk of ALS/FTD, we developed an online research platform, “Light The Way”, to offer those on this journey the education, counseling, peer support, and connections to research that they need. Initially deployed in Summer of 2023 in the United States and United Kingdom in both English language and US Spanish, Light The Way has a dynamic flow of content and modules that are responsive to each individual’s path and preferences. All participants are offered signposting to local ALS/FTD centers of excellence for care, signposting to peer-led social support, and a “Genetics 101” education program written by a genetic counselor. For those who want to understand their personal risks of developing ALS/FTD (if pre-symptomatic) or understand the cause of their condition (if already diagnosed), participants are also offered 1:1 pre-test genetic counseling, genetic testing via whole exome sequencing and PCR for repeat expansions covering over 40 genes, with 1:1 post-test genetic counseling for the return of results, all free of charge. A series of brief psychological distress screeners (PHQ-2, GAD-2, Hopelessness, perceived benefits / regrets) will be fielded longitudinally at baseline and at key milestones to describe the impact of the program. Individuals can share these results with their healthcare providers.

Results: Results will be presented for uptake, disposition, and distress characteristics at baseline. Initial funding has been allocated for 1,000 users of which up to 200 are anticipated to receive sequencing for ALS genes.

Conclusions: Genetic forms of ALS affect families far beyond local boundaries, and with the support of the community we will develop a sustainable platform to offer education, support, and fellowship to those in need.

270. LinkELA: ALS Telemedicine project in Barcelona to facilitate a multidisciplinary follow-up from the patient's home

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INTRODUCTION: Telemedicine (TM) contributes to bridge the gap between healthcare facilities and patients' homes with neuromuscular disease (NMD) because of mobility issues1. TM has proven to be useful and effective and therefore appreciated by both clinicians and patients2,3, especially in the context of COVID-19 in ALS4,5. Since there is no curative treatment, the approach to improve disease management and quality of life of patients is the implementation of multidisciplinary and palliative care7,8. It seems reasonable that multidisciplinary care should extend to the implementation of telehealth monitoring complementary to routine clinical visits in order to improve the patient's quality of life.

LinkELA is a patient-centered App, owned By Fundació Miquel Valls, which aims to improve the quality of life of ALS patients by offering a holistic and integrative follow-up, composed of different questionnaires typically managed by a multidisciplinary team.

METHODOLOGY: LinkELA is an app that has been developed by Doole Health, a spin-off of the Fight AIDS Foundation. A pilot was tested from December 2020 to March 2021 to define content, periodicity and useful tools for users.

On the one hand, the current version contains 4 questionnaires (pneumology symptoms, ALSFRS, ROADS, EAT-10) that patients have to fill in monthly or quarterly and the software analyzes patients answers to alert clinicians to problems.

On the other hand, physicians also have access to the tool and monitor data recorded by patients such as nutrition, ventilation, mobility, or emotional parameters. The alarm system helps physicians identify pronounced progression by color-coding to grade the urgency, avoiding unnecessary trips and visits to the hospital, but ensuring continuity of care by being closer to the patient's home.

All ALS at Hospital Universitari de Bellvitge and Hospital del Mar patients according to their digital preferences, have been offered to download the app after signing an Informed consent.

RESULTS: The app was launched in March 2022 and there are currently 18 active users out of a total of 58 registered users (including users during the test period) with a mean follow-up duration of 6 months (1,3-11,2 months). The mean age of the total population is 63.5 years (37-84); 66% are females.

A total of 1327 questionnaires have been sent during the first year follow-up, with a response rate of 38% overall and 35%; 37% and 39% for the ALSFRS, EAT-10 and both ROADS and the pneumology questionnaire, respectively. The mean ALSFRS-R values do not differ significantly between the standard version and the self-administered version (32 vs 30), and the intraclass correlation coefficient (ICC) is 0.85 for the total score: 0,91 for the fine gross domain, 0,89 for the gross motor domain and 0,71 for the bulbar and the respiratory. Women or patients aged 60-70 show higher ICC, 0,90 and 0,89 respectively. Regarding King's Stage, stage 3 classification shows an ICC of 0,89 while MiTos shows an ICC below 0,75 in all stages. A worse ICC has found for non-PEG carriers, but only 3 patients have been included in this group.

The alarm system has generated 162 notifications: 23% come from the ALS-FRS, 20% from the EAT-10 and 54% from the pneumology questionnaire (no alarms for ROADS). In addition, most alarms have coded as red or severe (52%) followed by orange/ medium and yellow or mild (27% and 19% respectively).

CONCLUSIONS: Agreement between the self-administered and standard ALS-FRS, patient acceptance and alarm system functionality are good after the nearly first year of using the tool. Thus, patients and physicians accept telemedicine as a complementary monitoring tool. However, it is important to further evaluate the application and adapt it to the needs if necessary, to maintain and develop the 4 main pillars of LinkELA based on communication, information, follow-up and adherence of ALS patients. Larger sample and further follow-up are needed to confirm these results.

271. Lipid metabolism in FTD-ALS spectrum: A pilot study

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INTRODUCTION: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are a clinical and neuropathological continuum. Clinical observations, such as association of sporadic ALS to elite athletes, or phenotypical variability among siblings with same TARDBP or C9ORF72 pedigree, suggest environmental factors may affect the course of disease. The metabolism of a particular person might be playing a role in this process. We aim to study the association of lipid metabolic markers in a cohort of patients with FTD, ALS, and healthy controls.

METHODS: We are undergoing a prospective study on ALS, behavioral variant of FTD (bvFTD), mixed forms FTD-ALS, and healthy controls. The protocol includes clinical data, a survey about previous lifestyle habits (eating behavior, physical exercise, morphotype, fat body distribution), a cognitive and neuropsychiatric assessment focusing on executive functions, social cognition and apathy; anthropometric and bio-impedance measures, and blood samples for metabolic analyses and genetics for common bvFTD and ALS genes (C9ORF72, TARDBP, SOD1, FUS, PGRN, MAPT). Patients are followed up after 12 months, and clinical data, cognitive batteries, blood samplings and anthropometries are taken a second time.

RESULTS: To-date, 57 participants were recruited: 30 patients (16 ALS, 11 bvFTD, 3 FTD-ALS), most of them sporadic (1 SOD1-ALS, 1 C9ORF72-FTD-ALS) and 27 healthy controls. We found preliminary differences in food preference and behavior, cognitive profiles, lipid profile, and other variables between FTD and ALS. Lipid metabolic profile and eating habits in the FTD-ALS patients seem similar to FTD.

CONCLUSIONS: This pilot study helped validate the protocol and pipeline to study the metabolic association with ALS and FTD patients and we are currently recruiting a higher number of patients
to complete a larger study.

272. Longitudinal cognitive assessment using the Cumulus home-based EEG platform in ALS and FTD.

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Background: People with ALS (pwALS) and/or FTD (pwFTD) often experience cognitive and/or behavioural change. However, detection can be confounded in one-off clinical/research visits due to factors such as fatigue and testing anxiety. A longitudinal home-based approach is more ecologically valid and provides less variable estimates of true performance. The Cumulus Neuroscience platform, consisting of a suite of tablet-based functional assessments, with synchronous portable dry electroencephalography (EEG), provides a comprehensive package to enable this approach.

Aims: To assess the feasibility of the Cumulus platform in pwALS, pwFTD and healthy controls; and to examine cognitive functioning using both the Cumulus platform and full neuropsychological assessment.

Methods: PwALS, pwFTD and healthy controls were recruited to a longitudinal study
of cognition over 8 months. Participants complete a full neuropsychological assessment at baseline, 4 months, and 8 months. Concurrently, participants complete three 25-minute Cumulus platform sessions every 2 weeks, measuring emotion recognition, inhibition, working memory, visuospatial memory, and synchronous EEG. Feasibility was assessed in terms of adherence rate, and the %bof successfully uploaded sessions. ALS, FTD and healthy control groups were compared on a subset of Cumulus (averaged over first 6 weeks) and full battery measures of cognitive functioning at baseline using one-way ANOVAs.

Results: Eleven pwALS (2 withdrawals), 8 pwFTD and 10 age- and education-matched controls have been enrolled. The adherence rate was 47.29% for pwALS, 68.92% for pwFTD and 68.17% for healthy controls, withall groups % of successfully completed sessions over >85%. On full battery baseline, a significant main effect for group status was observed on ECAS total, $F(2,20) = 3.74$, $p<.05$, $- 2 = 0.27$, and Digit Span forward, $F(2,19) = 4.65$, $p<.05$, $- 2 = 0.33$. On the Cumulus platform, a significant main effect was observed on the Digit Symbol Swap task, $F(2,17) = 6.39$, $p<.01$, $- 2 = 0.43$. For both Cumulus and full battery measures, pwFTD scored lowest on mean performance, then pwALS, then controls.

Conclusions: Preliminary evidence suggests that the Cumulus neurocognitive platform is feasible for pwALS and pwFTD and can identify cognitive deficits in these groups. Further research will establish how comparable the Cumulus platform is with gold-standard neuropsychological assessment and its sensitivity in detecting cognitive decline.

273. Longitudinal remote monitoring of nocturnal heart-rate and respiration in people living with amyotrophic lateral sclerosis

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Introduction

The ability to objectively monitor progressive symptoms of amyotrophic lateral sclerosis (ALS) is severely limited. Recent technological developments offer the ability to remotely and passively monitor patients living with ALS's nocturnal heart and respiration rate from within their homes using an under-mattress sleep sensor. The ability to remotely monitor patients' physiology provides a way to develop new digital biomarkers to monitor disease progression. These digital biomarkers may provide ways to improve patient's care and clinical decision making. Nocturnal digital biomarkers may provide sensitive ways of determining the effect of new therapies.

Methods

In this prospective observational study, 20 patients with ALS will be monitored over 12 months. Patients will have a Withings Sleep Analyzer fitted under their mattress, which passively records their sleep along with heart-rate and respiration-rate. Patient's will also complete a monthly ALSFRS-r assessment. Longitudinal changes in hear-rate and respiration will be analysed using linear-mixed effects models and will be compared to healthy case-matched controls.

Aims:

1. The aim of the study is to evaluate the feasibility of using a sleep sensor for remote monitoring of nocturnal heart rate and respiration in people living with ALS.

2. Determine whether longitudinal changes in nocturnal heart-rate and respiration can serve as digital biomarkers for disease progression in ALS.

3. Determine the natural history of longitudinal nocturnal physiology in patients with ALS.

Introduction: The ability to objectively monitoring progressive symptoms of amyotrophic lateral sclerosis is (ALS) is crucial for optimising patient care and evaluating therapeutic interventions. Recent technological advancements enable remote, passive monitoring of nocturnal heart-rate and respiration in patients with ALS using an under-mattress sleep sensor. Developing digital biomarkers for monitoring disease progression has the potential to improve clinical decision-making and evaluate the efficacy of novel therapies.

274. Long-term follow-up of five Turkish families with UBQLN2 mutations

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BACKGROUND: Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease (MND), caused by degeneration of upper and lower motor neurons. UBQLN2 gene, which encodes ubiquilin-2, a member of the ubiquitin-like protein family, has been associated with X-linked ALS.

HYPOTHESIS: In this study, we aim to further define the phenotypic characteristics of the UBQLN2 mutations in the ALS patients from Turkey.

PATIENTS AND METHODS: Clinical and genetic findings of six patients and five asymptomatic carriers with UBQLN2 variants from five unrelated Turkish families diagnosed with possible, probable, or definite ALS by El Escorial criteria at the Department of Neurology, Istanbul Medical Faculty were evaluated. The samples were collected with the approval of the relevant institutional ethics boards, and informed written consent was obtained from each participant.

RESULTS: Two of the symptomatic and four of the asymptomatic patients were female. The mean age of onset was 20.7±7.84 (ranges 12 and 36) years. The mean age of asymptomatic patients were 48.2 (range 35-76) years. None of our patients had dementia. Three of them presented with weakness and muscle wasting of one hand, three with bulbar dysfunction symptoms, and one with foot drop. Four of our patients had prominent lower motor neuron signs at the onset of the disease. Five patients died during follow-up because of respiratory complications, mean time to death after the onset of disease was 8.4 years (ranges 4-14 years). Four distinct missense variants (P506S, P525S, M392I, S340I) were found in our families.

CONCLUSIONS: This is the largest cohort from Turkey, indicating genotypic and phenotypic heterogeneity of UBQLN2 mutations. The S340I change was identified in 3 unrelated families from Turkey previously. M392I variant was found in two of our patients with distinct phenotype (Madras MND and juvenile onset slowly progressive MND). Further analyses are needed to clarify the pathogenicity of the M392I variant of UBQLN2.

275. M102 as a therapeutic approach for ALS poised for patient stratification approaches

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Background: Therapeutics to treat ALS are desperately needed, with survival being improved by only a few months with the current drugs approved for use. ALS is known to involve several different contributing pathological mechanisms which can differ between individual patients and this might contribute to the heterogeneity observed in the patient population. It may be, therefore, beneficial to develop a patient stratification approach that allows the identification of which patients will respond to a particular treatment. We have previously shown that patient-derived astrocytes are toxic to motor neurons in co-culture, and that an antioxidant drug, called M102, can increase neuron survival with some patient's cells but not others.

Aims: Here we aimed to identify responders and non-responders to M102 in a large cohort of sporadic ALS patients, and to compare changes in gene expression which correlate with response.

Methods: We have directly reprogrammed 20 sporadic ALS patient fibroblast lines into induced-neuronal progenitor cells (iNPCs) and subsequently differentiated these into astrocytes.

Results: So far we have assessed the pathological characteristics of the astrocytes derived from 10 sALS patients. Using a combination of immunostaining and immunoblotting techniques, we have confirmed that M102 can induce activation of the NRF2 and HSF1 signalling pathways for up to 72h post-treatment. Importantly, M102 significantly reduces TDP-43 pathology by reducing the levels of TDP-43 fragments, while increasing full length protein. In addition to this, by co-culturing these astrocytes with motor neurons, we have identified responders and non-responders to M102. Transcriptomic profiles of these astrocytes and PBMCs from the same patients will be used to predict patient-specific response to M102.

276. Methods to test for causality in Amyotrophic Lateral Sclerosis: A Real-World Data example using ALS Natural History Study and MiToS Staging

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OBJECTIVE: This analysis aims to evaluate how studies collecting longitudinal data from ALS patients, such as the ALS Natural History Study (ALS-NHS), can be used to draw causal inference effects.

BACKGROUND: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that needs to be fully understood. Over the past two decades, research has increased exponentially, and several data have been collected. Therefore, the entire natural history of the disease is not fully understood.

DESIGN: We report how ALS-NHS can be used to define causal effect or describe association. We aimed to assess the causal relationship between survival in ALS with both MiToS respiratory domain and sex. ALS-NHS enrolls every patient at their respective site, and the breadth of data covers the full heterogeneity of ALS presentation and progression. The generous inclusion criteria create a dataset that is an excellent candidate for causal inference using matching algorithms to test the efficacy of interventions or putative factors arising from medical history, demographics, or other baseline characteristics at diagnosis.

The following four stages of the causal pipeline were used: i) conceptual stage (formulating the plausible causal question), ii) design stage (framing the causal question as if the analysis had been conducted as a randomized-blinded clinical trial), iii) statistical analysis (estimating and testing the null hypothesis of no effect of the intervention), and finally iv) results.

RESULTS: 1521 ALS patients over 18 years of age were extracted from the ALS-NHS. To

test the validity of the methodology, we attempted to replicate a positive and negative control, MiToS respiratory domain and sex, respectively. Our analysis showed that ALS patients with a higher MiToS Breathing domain score at baseline have lower survival (p=0.01, 11 months on average). Regarding causality between survival in ALS patients and sex, we cannot reject the null hypothesis of a difference in survival for male versus female patients.

CONCLUSIONS: Natural history studies can be used to estimate the causal effects through the four-stage approach. The power of these studies can be compared to a classic randomized analysis. Given the high variability of the data due to the lack of inclusion/exclusion criteria, these clinic data can be used as a control group to validate treatment efficacy for patients.

277. Modeling pathological spread through the structural connectome in the FTLD spectrum

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Objective. To explore the relationship between network vulnerability and atrophy progression in patients within the frontotemporal lobar degeneration (FTLD) spectrum, using Network Diffusion Model (NDM) of pathology spread.

Materials. Thirty-four behavioural-variant frontotemporal dementia (bvFTD), 11 semantic-variant primary progressive aphasia (svPPA) and 11 nonfluent/agrammatic-variant primary progressive aphasia (nfvPPA) patients underwent longitudinal T1-weighted MRI. Forty-eight young healthy subjects (20-31 years) underwent multi-shell diffusion MRI scan. NDM was developed to assess whether the progression of FTLD pathology might be modeled by a spreading process, originating from a seed and then proceeding through the healthy structural connectome. The connectivity measures used to create the structural connectome were fractional anisotropy (FA) and intra-cellular volume fraction (ICVF). Three disease epicenters were identified from the peaks of atrophy of each FTLD variant: right orbitofrontal cortex (bvFTD), left inferior temporal gyrus (svPPA), and left supplementary motor area (nfvPPA). Correlations were tested between atrophic changes estimated by NDM and those empirically obtained in FTLD patients over a follow-up of 24 months.

Results. In the case of bvFTD, NDM showed an early spread to frontal lobe and basal ganglia (6 months) and to right sensorimotor, parietal, temporal and occipital lobes (12 months), with an involvement of the left hemisphere between 18 and 24 months. In svPPA, NDM predictive maps in young controls suggested an early spread of pathology to the left occipital (6 months) and inferior frontal lobe (12 months). At 18 months, left parietal lobe would be reached, whereas only few regions in the right parietal and occipital lobes would be affected at 24 months. In nfvPPA, NDM predicted a pathology spread through all brain regions, except for the occipital lobe, which would be involved after 12 months. NDM-predicted atrophy of each region was positively correlated to longitudinal atrophy empirically observed in all three FTLD variants. Overall, NDM applied on ICVF connectome provided higher correlation values relative to NDM applied on FA maps.

Conclusion. The NDM implementation to cross-sectional structural connectome is a valuable tool to predict atrophy patterns and pathology spreading in FTLD variants.

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278. Molecular dynamics analysis of Superoxide Dismutase 1 mutations suggests decoupling between mechanisms underlying ALS onset and progression

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Mutations in the superoxide dismutase 1 (SOD1) gene are the second most common known cause of ALS and are generally considered to cause ALS via a gain-of-function mechanism. SOD1 variants express high phenotypic variability and over 200 have been reported in people with ALS. Investigating how different SOD1 variants affect the protein dynamics might help in understanding their pathogenic mechanism and explaining their heterogeneous clinical presentation. It was previously proposed that variants can be broadly classified in two groups, 'wild-type like' (WTL) and 'metal binding region' (MBR) variants, based on their structural location and biophysical properties and that MBR variants, but not WTL variants, are associated with a loss of SOD1 enzymatic activity. In this study we used molecular dynamics and large clinical datasets to characterise the differences in the structural and dynamic behaviour of WTL and MBR variants with respect to the wild-type SOD1, and how such differences influence the ALS clinical phenotype.

Our study identified marked structural differences, some of which are observed in both variant groups, while others are group specific. Moreover, applying graph theory to a network representation of the proteins, we identified differences in the intramolecular contacts of the two classes of variants. Finally, collecting clinical data of approximately 500 SOD1 ALS patients carrying variants from both classes, we showed that the survival time of patients carrying MBR variants (the ones that contribute to loss of SOD1 function), is substantially longer (~6 years median difference, $p < 0.001$) with respect to patients with a WTL variant [1]. In conclusion, our study highlights key differences in the dynamic behaviour of the WTL and MBR SOD1 variants, and wild-type SOD1 at an atomic and molecular level. We identified interesting structural features that could be further investigated to explain the associated phenotypic variability. Our structural results support the hypothesis of a decoupling between mechanisms of onset and progression of SOD1 ALS and align with recent clinical evidence [2]. Moreover, they support the involvement of loss-of-function of SOD1 with the disease progression [3].

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279. Motor Neuron Disease Systematic Multi-Arm Adaptive Randomised Trial (MND-SMART): Delivering innovation in clinical trials for MND/ALS

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Definitive evaluation of promising therapies for motor neuron disease (MND) requires a novel approach to trial design. Multi-arm multi-stage (MAMS) platform randomised phase III trials allow: (i) simultaneous definitive evaluation of multiple treatment arms against a single control group, (ii) early cessation of treatments showing no sign of activity, and (iii) addition of new arms in a 'continuous' trial platform. These features deliver efficiencies in time, cost and sample size requirements when compared with serial two-arm studies. MND-SMART (NCT04302870) is globally amongst the first MAMS platform phase III double-blind, placebo-controlled trials launched for a neurodegenerative disease. Commencing in 2/20, it has recruited > 500 participants randomised across 19 UK sites.

MND-SMART currently assesses the individual efficacy of two interventional arms added to standard of care—memantine and trazodone—against standard of care plus a single contemporaneous placebo. The co-primary outcome measures are ALS-FRS(R) and survival. ALS-FRS(R) comparisons are conducted in three stages, with predefined criteria for futility at the end of stages 1 and 2. MND-SMART's broad inclusion criteria and co-production with people with MND (pwMND) have enabled wide participation and a low withdrawal rate. The analysis plan permits pre-determined subgroup analyses. Stage 1 analysis, completed after 50 participants/arm completed 6 months of treatment, occurred in 2022. Both interventional arms passed the futility analysis. Stage 2 analysis will be undertaken when 100 participants/arm have completed 12 months of treatment (anticipated Summer 2023).

The next steps also include widening access through opening new UK sites, evaluating more drugs (selected through an integrated human 'stem-cell-in silico-in vivo' drug discovery platform), enhanced bio-banking of samples enabling reverse translation research, and deployment of novel digital tools for high-frequency remote data acquisition. In summary, MND-SMART showcases innovation in trial design that, through inter-disciplinary collaboration, seeks to improve outcomes for pwMND.

280. Navigating through a changing sea: The emotional journey of current family carers of people living with MND

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Being a family carer of someone living with Motor Neurone Disease (MND) can have emotional consequences for the carer due to the constant changes and losses happening due to MND. Carers' feelings and emotional wellbeing might impact their ability to perform their own everyday activities and their caring role, yet little is known about how carers manage their emotions during the trajectory of the disease.

In-depth interviews were conducted with fourteen current family carers of people living with MND living in the UK. Interviews were either online or face-to-face, audio or video recorded and professionally transcribed verbatim. Data collected were analysed inductively with reflexive thematic analysis, within an interpretive descriptive framework.

The analysis produced three themes. Being drifted out to sea reflected the emotional impact the diagnosis had on carers, such as emotions of shock, devastation and hopelessness, and how they transited this new reality. Learning to navigate in a stormy sea encompassed how carers experienced and felt about the everyday changes that MND brought and how they started to adapt and face these changes to integrate MND into their lives and be able to continue with everyday activities and supporting the person with MND. Controlling the rudder in a choppy sea captured how carers identified and used individual approaches to cope emotionally with the continuous and unforeseen changes and maintain their emotional wellbeing through the progression of the disease in a narrowed world.

These findings suggest that carers experience different emotions during the trajectory of MND. With diagnosis, carers experience a substantial emotional destabilisation, which gradually eases as carers begin to cope emotionally by adapting and accepting the changes and losses happening due to MND. As the disease progresses, carers identify approaches that best work for them to manage their emotions and feelings (e.g., not thinking about the future, looking for informal support, living day by day).

This study identified destabilising factors and meaningful strategies used by family carers of people with MND for managing their emotions and feelings. These understandings of how carers re- construct their emotional life around MND could help inform future practice and research to better support carers of this population.

281. Neurofilament light chain response during therapy with Tofersen in SOD1-related ALS – treatment experience in clinical practice.

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Introduction/Aims: In amyotrophic lateral sclerosis (ALS) caused by superoxide dismutase 1 (SOD1) gene mutations (SOD1-ALS), the antisense oligonucleotide tofersen had been investigated in a phase 3 study (VALOR) and subsequently introduced in an expanded access program. This study assesses neurofilament light chain (NfL) before and during tofersen treatment.

Methods: In six SOD1-ALS patients treated with tofersen at three specialized ALS centers in Germany, NfL in cerebrospinal fluid (CSF-NfL) and/or serum (sNfL), the ALS Functional Rating Scale–Revised (ALSFRS-R), and ALS progression rate (ALS-PR), defined by monthly decline of ALSFRS-R, were investigated.

Results: Three of six SOD1-ALS patients reported a negative family history. Three patients harbored a homozygous c.272A>C, p.(Asp91Ala) mutation. These and two other patients showed slower progressing ALS (defined by ALS-PR <0.9) whereas one patient demonstrated rapidly progressing ALS (ALS-PR=2.66). Mean treatment duration was 6.5 months (range 5-8). In all patients, NfL decreased (mean CSF-NfL -66%, range -52 to -86%, mean sNfL -62%, range -36 to -84%). sNfL at 5 months of tofersen was significantly reduced compared to the measurement closest before treatment (p=0.017). ALS-PR decreased in two patients whereas no changes in ALSFRS-R were observed in four participants who had very low ALS-PR or ALSFRS-R values before treatment.

Discussion: In this case series, the significant NfL decline following tofersen treatment confirmed its value as a response biomarker in an expanded clinical spectrum of SOD1-ALS. Given the previously reported strong correlation between sNfL and ALS progression, the NfL treatment response contributes to the notion of disease-modifying activity of tofersen.

282. Neurological Soft Signs in unaffected relatives of people with familial ALS: an expanding endophenotype of neurological dysfunction in ALS kindreds

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Background:

Neurological soft signs (NSS) are non-disabling abnormalities of coordination, sensory motor integration, complex motor sequencing, and reflexes. Cognitive endophenotypes have been identified in unaffected first and second-degree relatives of people with familial ALS (fALS)(1), indicating an underlying shared vulnerability amongst these kindreds. The psychiatric literature has long established an endophenotypic pattern of NSS in people with schizophrenia and their unaffected relatives(2,3), but NSS have not been studied in this capacity in neurological literature. This study aims to investigate NSS as a candidate endophenotype in fALS.

Methods:

First and second-degree relatives of fALS patients and healthy controls underwent a structured NSS examination comprising an assessment of primitive and deep tendon reflexes, mirror movements, sensory integration, and motor coordination, sequencing, and planning. Higher scores indicated worse performance and a greater burden of NSS.

Results:

A preliminary sample of 24 fALS relatives and 19 healthy controls were well matched for age ($p = 0.075$) and gender ($p=0.16$). FALS relatives had higher total NSS scores compared with controls ($p<0.0001$). In particular fALS relatives scored significantly worse than controls on tests of finger gnosis ($p=0.04$) and graphaesthesia ($p=0.04$). Total sensory integration scores (combining graphaesthesia, stereognosis, finger gnosis, and left-right disorientation) were worse in fALS relatives than in controls ($p=0.008$). A significantly higher proportion of fALS relatives demonstrated hyperreflexia compared with controls (79% vs. 26%, $X = 12.01$, $p = 0.001$).

Conclusion:

A high burden of NSS may constitute a potential endophenotype in first and second-degree relatives of people with fALS. This novel finding endorses previous research identifying a cognitive endophenotype in this cohort and points to an overall vulnerability amongst these kindreds that has yet to be fully understood.

1 Costello et al., 2018. ; 2 Neelam et al., 2011.; 3 Xu et al., 2016.

283. Non-Invasive Mechanical Ventilation reduces the Motor Decline in Amyotrophic Lateral Sclerosis

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Background

Non-invasive mechanical ventilation (NIMV) improves Amyotrophic Lateral Sclerosis (ALS) quality of life and survival. However, data about its effect on disease progression are still lacking.

Objective

To test whether NIMV use changed the rate of functional decline among ALS patients.

Methods

In this retrospective observational study, we included 465 ALS patients followed up at the ALS Center in Turin, Italy, who underwent NIMV during the disease course. The primary outcome was the change in functional decline after NIMV initiation when adjusting for covariates. Functional decline was based on the non-respiratory items of the ALS Functional Rating Score – Revised (ALSFRS-R).

Results

A slower rate of functional decline followed NIMV initiation (estimate improvement 0.13, 95% CI 0.11 to 0.16, $p<0.001$) regardless of sex, age at diagnosis, and disease duration before NIMV initiation. Indeed, the disease stage at NIMV initiation (early or advanced) did not influence the results. Respiratory support exerts its slowing effect mainly on the progression of spinal motor function.

Conclusions

We prove that NIMV influences the rate of motor progression in ALS. This result was not a consequence of the ALSFRS-R floor effect. The functional decline slowed after starting NIMV independently of the site of disease onset. Our results reinforce the importance of not delaying NIMV initiation in all ALS patients. NIMV-induced slowing of disease progression should also be accounted for when evaluating clinical trial outcomes.

284. Oral ketone bodies as novel therapy to compensate the energy deficit in patients with Amyotrophic Lateral Sclerosis (KETO-ALS)

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Scientific Background: Weight loss is a common phenomenon and a negative prognostic marker in ALS. Previous studies have shown hypermetabolism in both ALS mice models and patients. The pathogenic background of hypermetabolism remains currently unclear.

Ketone bodies constitute an extremely high-energetic substrate which can cross the blood brain barrier via a specific, physiological transporter. Moreover, they provide a significant higher amount of ATP per mole compared to glucose. Ketogenic diet has already shown beneficial effects in other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease and was also effective in a mouse model of ALS, where mice treated with a ketogenic diet showed a significant slower decline of motor function and also gained body weight compared to controls. Due to dysphagia and the need of a long-term change of eating habits, the direct application of ketone bodies may constitute an effective and easy-to-apply alternative compared to a ketogenic diet, which significantly increase ketone body serum levels, sustaine for several hours in humans and is completely safe and well tolerable.

Hypothesis: Therefore, we hypothesize that the energy deficit in ALS can be targeted by administering oral ketone bodies as a high-energetic substrate, and that this intervention may slow down disease progression and lower NfL serum levels.

Preliminary Study: Subsequently, we conducted an individualized treatment with ketone esters in 2 patients over 2 days and found that β-HB serum levels increased significantly up to maximum values of 3.7 mg/dl about 10 minutes after intake, corresponding to about the 8-fold baseline concentration. Over the following time, β-HB serum levels decreased again and reached baseline levels 1–2 hours before the next intake.

Study design: In our study (KETO-ALS) 76 patients will be recruited, 38 per treatment arm. The study is designed as double-blind randomized controlled trial. Primary endpoint is the change of NfL serum levels after 6 months compared to baseline. The changes will be measured as individual NfL slope from baseline to 6 months (loss of NfL per month), calculated for each patient.

285. Outreach to Spanish-speaking populations in Latin America to improve participation in clinical trials offered at the Clinical Research Unit (MNI)

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The importance of clinical trials is crucial to find an effective treatment for Amyotrophic Lateral Sclerosis (ALS) and, eventually, its cure.[1]There is a clear need for clinical trials in ALS in Latin America [2]. Despite that fact, only less than three percent of the clinical trials in ALS are based in that area, as of today. (Clinicaltrials.gov.). The lack of trials offered in Latin America causes different challenges for patients living in the area who are interested in participating.[3, 4] The absence of Latin American patients participating in Clinical trials in other parts of the world is a combination of many factors. [5] An important limitation for patients in those countries is the conversion rate to the local currency. Language, along with cultural differences, are often other factors of concern for the patients. Another important factor is the lack of awareness by local health professionals about such trials (salud-america.org). In an attempt to improve Latin American patient access, an intensive communication network has been established with neurologists, family doctors, genetic counselors, and patients in Argentina, Mexico, Bolivia, Uruguay, Chile, Colombia, Venezuela, and Brazil to inform them about ongoing trials and their benefits. The objective of this project is to raise awareness about the Clinical Research Unit (CRU)at the Montreal Neurological Hospital, and the trials run in the institution and to offer the possibility of enrollment, in their native language. Information about the trials and their sponsorship program is vital in these cases. By using this communication network, we hope to build a stronger relationship between the CRU and Latin America to increase the reach of Latin American populations to the trials and their potential benefit, until they are offered locally.

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286. Patient and technical feasibility of real-world sampling of cognition and functional neurophysiology in ALS and FTD

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Background Clinic-based assessments can be effective in tracking disease progression in clinical research, but do not scale to support public health needs or large-scale real-world studies. Novel digital platforms are scalable but their usability must be demonstrated in patient populations suffering from cognitive and motor impairments, and behavioral changes.

Aim Characterization of the feasibility of the Cumulus neurocognitive platform for repeated at-home use by FTD and ALS patients, and age-matched controls.

Methods Patients with ALS/FTD, and controls were recruited for an 8-month repeated sampling study in Ireland. Participants are requested to complete three 25-min sessions every 2 weeks in the home, using gamified tasks (affective processing, language, executive, memory and motor functions) while synchronized EEG was recorded using a self-applied dry-sensor headset. Key behavioural and EEG endpoints were evaluated cross-sectionally based on data from the first five sessions for each assessment.

Results 10 controls, 8 FTD and 11 ALS were enrolled in this study, with 3 withdrawals.

Adherence was 47.3% for the ALS group (mean age=62.9, SD=8.8), 68.9% for the FTD group (mean age=63, SD=8.5), and 68.2% for the controls (mean age=60.4, SD=9.2). Behavioral data analyses revealed a main effect of group in the travel time of the Psychomotor task ($p<0.001$), in the number of successful responses in the Digit Symbol Swap task ($p=0.002$) and in the speech rate of a picture description task ($p=0.004$). Post-hoc tests showed that the FTD had a longer travel time (Psychomotor) and fewer correct responses (Digit Symbol Swap) than controls and ALS. Additionally, speech rate was higher in controls compared to ALS and FTD. No main effect of group was found in the associative learning task, nor in the total speech duration of the picture description task. Visual inspection of the P300 event-related potential (ERP) suggests a reduction of amplitude and increase in latency of this ERP in the FTD and ALS group at key centro-parietal sites (Pz, CPz).

Conclusions Interim analyses from this first-in-class study suggest that older individuals, and those with FTD and ALS are capable and willing to participate in home-based studies with intensive repeated sampling protocols. Initial examination of the data suggests that digital remote measurements can separate the groups, constituting positive initial evidence for task validity and technical feasibility.

287. Patient self-reported symptoms and assessment of the most impaired activities in a large multicenter ALS cohort

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Introduction: Amyotrophic lateral sclerosis (ALS) affects individuals differently during the course of the disease. Our aim was to investigate the most significant symptoms from the patient's perspective and identify the essential and preferred activities that are most affected by the disease.

Methods: This cross-sectional study was carried out since 9/2021 at 13 ALS-centers across Germany, as part of the ID-ALS study. We designed a questionnaire, derived from the Measure Yourself Medical Outcome Profile (MYMOP), which allowed patients to indicate the most relevant symptoms experienced in the different types of ALS and at different stages of the disease. It included a ranking of a preferred activity that is most affected by ALS and the overall well-being on a scale of 0 to 6 (best to worst). The ALSFRS-R was documented. Epidemiologic data, disease duration, and specific ALS variants were also collected. Subsequently, symptoms and activities were categorized and the patient cohort was grouped according to disease severity, duration of disease, age, gender, and ALS variants.

Findings: Over 1200 questionnaires were collected, of which 1109 were included into the analysis (649 male, 460 female; 1.4:1). The median age was 63 years. Categorization of symptoms revealed respiratory, bulbar, axial, upper and lower extremity function, mental, and generalized symptoms (e.g., fasciculation). The vast majority reported symp-

toms of the arms, legs, and bulbar dysfunction. Activities have been classified into the following domains: Work, hobby and recreation, walking and mobility, sport and exercise, social participation (including verbal interaction), self-care and independence. Activities demanding movement were the most frequently named (48%), followed by recreation and hobby (21%). However, patients' well-being correlated strongest with the categories social participation as well as self-care and independence ($p<0.001$).

Conclusion :Patient-reported symptoms, their individual assessment and classification of impaired activities enables characterization of ALS from the perspective of the affected individuals. This study revealed the most commonly reported symptoms and activities relate to specific and general movement, with a major impact on recreation, social activities, interaction, and desire for independence as proximate consequences of the disease. Patient preferences should be considered when assessing clinical relevance of potential therapies.

288. Patients' and caregivers' views of multidimensional and palliative care in amyotrophic lateral sclerosis – protocol of a German multicentre study

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Amyotrophic lateral sclerosis (ALS) leads to a progressive loss of physical functioning, which also results in high psychosocial burden and organizational challenges related to medical care and aids. A multidimensional and multiprofessional care is advised in order to meet the complex requirements of this disease. In Germany, medical care structures may not fulfil these high requirements, since non-medical services such as psychological support or social counselling are not regularly included in care procedures for ALS patients. Specialised palliative care is not a stan-

dard and commonly restricted to the last weeks of life. Additionally, it is well known that caregivers of ALS patients are highly burdened, but there is still a lack of support services for them.

By means of a cross-sectional, multicentre survey, we investigate patients' and caregivers' perception of medical care for ALS provided in Germany - with particular regard to psychosocial and palliative aspects. The extent to which physical, psychological, social, spiritual, practical and informational needs are subjectively met will be associated to mental wellbeing, subjective quality of life, attitudes towards life-sustaining measures and physician-assisted suicide, as well as caregiver burden.

The study aims to recruit 500 participants from nationwide ALS-centres, in order to derive comprehensive conclusions for Germany. It is intended to provide data-based starting points on how care of the highly vulnerable ALS patients and their caregivers can be improved in line with their needs.

Currently, study planning and initiation is completed. A total of 26 centres - mostly acquired via the clinical and scientific German Network for Motoneuron Diseases (MND-Net) - will take part in the project, 13 of which already started recruitment.

The study is supported by the Deutsche Gesellschaft für Muskelkranke e.V., the largest self-help association for neuromuscular diseases in Germany.

289. People at Risk of Genetic ALS/FTD Want Pre-Symptomatic Treatment: Results of an Online Survey

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Objective: People at risk of genetic ALS/FTD connect online, volunteer for research, and advocate for their community. In preparation for a FDA workshop, we sought to understand how our community feels about taking potential therapies during the pre-symptomatic period.

Methods: Between September 30th, 2022 and October 21st, 2022, an anonymous survey was distributed via email, social media, and online communities. Topics included demographics, perceived disease risk, levels of desire for pre-symptomatic treatment, and levels of side effects or inconveniences considered tolerable against potential levels of efficacy.

Results: Responses were collected from 174 eligible respondents on the desire for presymptomatic treatment; most were female (72.9%), and White/Caucasian (95.3%). Most respondents (120/174, 69.0%) would be interested in treatment as early as possible, even prior to having clinical signs or symptoms of ALS/FTD. A minority (41/174 (23.6%) would be interested only at the first biological signs of disease activity (i.e., a biomarker signal) and just a fraction (9/174, 5.2%) were interested only when symptoms first appear. Only a handful (4/174 2.3%) were not interested in presymptomatic treatment entirely. If a regulatory agency approved a treatment for ALS/FTD in trials conducted only with symptomatic patients, then 165/170 (97.1%) of eligible respondents said they would consider taking it pre-symptomatically.

Conclusions: People at risk of ALS/FTD have a strong desire for pre-symptomatic treatment and are able to balance potential hazards of treatment against the potential for efficacy.

290. Peripheral immune signatures in ALS: a population-based study

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Introduction. Systemic inflammation has been proposed as a mechanism in Amyotrophic Lateral Sclerosis (ALS). Recent evidence shows that an increased inflammatory status correlates with survival in ALS. Still, comprehensive data on ALS patients' innate and adaptive immune responses and their effect on the clinical phenotype are lacking.

Objective. To investigate the role and characteristics of systemic immunity in a population-based ALS cohort using available hematological indexes that reflect changes in innate and adaptive immunity.

Design. We collected the complete blood count (CBC) at diagnosis in ALS patients from the Piemonte and Valle d'Aosta Register for ALS (PARALS) from 2007 to 2019. Demographic and clinical data were collected using registry data. Leukocytes populations, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII) and lymphocyte-to-monocyte ratio (LMR) were derived from CBC. All variables were analyzed for association with clinical features in the entire cohort and then in sex- and age-based subgroups. Logistic, linear and Cox regression models were used as appropriate.

Results. After adjustment for relevant covariates, neutrophils (p=0.001) and markers of increased innate immunity (NLR, p=0.008 and SII, p=0.006) were associated with a faster disease progression. Similarly, elevated innate immunity markers correlated with worse pulmonary function and shorter survival. Sex-based differences emerged, as the prognosis in women also correlated with a low lymphocyte (p=0.045) and a decreased LMR (p=0.013). ALS patients with cognitive impairment exhibited lower levels of monocytes (p=0.0415) and, although only in later-onset ALS (age at onset > 70 years), lower lymphocytes (p=0.006) and increased NLR (p=0.021) and SII (p=0.030).

Conclusions. Our results confirm that a dysregulated systemic immune system participates in ALS progression. More specifically, an elevated innate immune response is associated with faster progression and reduced survival. The immune response varied according to sex and age, thus prompting the speculation that involved immune pathways are patient-specific. Finally, we observed that ALS patients with greater cognitive impairment showed a reduction in monocytes count. Those data revealed that systemic inflammation plays a multifaceted role in ALS: further studies will help translate those findings into clinical practice or targeted treatment.

291. Phase 3b Extension Study Evaluating Superiority of Daily vs Approved On/Off Oral Edaravone Dosing in Patients With Amyotrophic Lateral Sclerosis

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INTRODUCTION: Intravenous edaravone (Radicava®/Radicut) was shown to slow the rate of physical functional decline in amyotrophic lateral sclerosis (ALS). This ongoing, multicenter, phase 3b, double-blind, parallel group, randomized extension study is evaluating 2 dosing regimens of Radicava ORS® (edaravone) oral suspension. Oral edaravone was approved by the United States Food and Drug Administration for use in patients with ALS in May 2022 and gained approval in late 2022 in Canada and Japan.

OBJECTIVES: Study MT-1186-A04 (NCT05151471) is evaluating and comparing the long-term safety, efficacy, and tolerability of 2 oral edaravone dosing regimens for up to an additional 48 weeks following the end of Study MT-1186-A02 in patients with ALS, comprising a total duration of up to 96 weeks.

METHODS: Study MT-1186-A04 will evaluate 2 dosing regimens of oral edaravone (105-mg dose): group 1 will have oral edaravone administered once daily for each 28-day cycle; group 2 will have oral edaravone administered for 10 days followed by placebo for 18 days in each 28-day cycle. Dosing in both groups will continue up to 48 weeks.

Study MT-1186-A04 is anticipated to include approximately 300 adult patients who have completed Study MT-1186-A02. The primary objective is to evaluate the efficacy of each dosing regimen based on the date of randomization in Study MT-1186-A02 to at least a 12-point Revised ALS Functional Rating Score decrease or death, whichever happens first, over the course of the study.

RESULTS: Ongoing

SUMMARY/CONCLUSION: This extension study will provide important information on the safety, efficacy, and tolerability of 2 oral edaravone dosing regimens in patients with ALS.

Sponsorship: Mitsubishi Tanabe Pharma America, Inc.

Acknowledgments: p-value communications provided editorial support.

292. Plasma exchange with albumin replacement modifies albumin fatty acid binding capacity in patients with amyotrophic lateral sclerosis (ALS)

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Background: Recently, two pilot clinical trials carried out on in Spain (13 patients; Povedano et al, 2021) and the US (15 patients; Obat et al, 2022) revisited plasma exchange (PE) with albumin replacement as a potential therapy for ALS, considering albumin's role in blood as a transporter with antioxidant, anti-inflammatory, and neuroprotective effects. Results indicated that the treatment was safe but showed a considerable heterogeneity in the response to treatment, with no overall clinical benefit. However, more than a half of the patients showed a slower than expected rate of decline at the end of treatment. Moreover, in the Spanish cohort, decreased albumin fatty acid binding capacity and lower 8-isoPGF2α (an oxidative stress marker) levels after PE procedures were observed. These results were deemed consistent with higher occupation of albumin fatty acid binding sites.

Aim: To confirm the PE-associated changes in albumin fatty acid binding capacity in plasma samples from the US cohort.

Methods: Plasma samples from 12 patients included in the US trial (NCT02872142) and 10 healthy controls (HC) were evaluated. Patients underwent 24 weeks of PE sessions using albumin 5% (Albutein, Grifols) as the replacement solution (3 weeks with 2 PE sessions per week followed by a 21-week with 1 PE session per week) plus a 24-week follow-up period. The binding capacity of albumin to fatty acids was determined by electronic paramagnetic resonance, incubating samples with increasing concentrations of the spin probe 16-doxylstearic acid and expressing the results by the dissociation constant (Kd). Results were expressed as median and IQR.

Results: In comparison to HC, albumin from ALS patients at baseline presented similar binding capacity to fatty acids (ALS 1.2 [0.9-1.8] vs HC 1.2 [0.6-1.8], p=0.50). Albumin binding capacity to fatty acids decreased just after selected PE (post vs pre samples: PE#7 and PE#15: p=0.01; PE#27: p=0.02) and it returned to baseline level at the end of the treatment (baseline 1.2 [0.9-1.8] vs follow-up visit at week 36 1.6 [1.1-2.1], p=0.54).

Conclusion: Decreased albumin fatty acid binding capacity after PE procedures in ALS patients was confirmed, in agreement to the findings with the Spanish cohort. The associated reduction of the lipoperoxidation marker 8-iso-PGF2α levels described in the Spanish study may reflect binding by albumin to lipidic substances mobilized during PE.Further studies to validate this hypothesis are warranted.

293. Plasma neurofilament analysis in VITALITY-ALS

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Background: Neurofilaments (Nf) are major constituents of the neuronal cytoskeleton. Nf light chain (NfL) and phosphorylated Nf heavy chain (pNfH) are potential biomarkers of ALS disease progression. Nf are released from degenerating neurons and can be measured in plasma or cerebrospinal fluid. VITALITY-ALS (NCT02496767) was a 48-week, randomized, double blind, placebo-controlled clinical trial of tirasemtiv in people with ALS. Plasma was collected at baseline (BL) and every 8 weeks with Nf measurements available from 101 placebo and 161 tirasemtiv treated subjects. Published results found tirasemtiv did not show an effect on vital capacity, ALSFRS-R or muscle strength [1].

Objectives: Compare BL Nf from VITALITY-ALS by treatment, clinical characteristics, and time.

Methods: Plasma NfL and pNfH were measured and compared by treatment, clinical characteristics, and time using a Mixed Model for Repeated Measures with BL value adjustment. Pearson Correlation Coefficients were calculated to evaluate correlations between pre-study rate of disease progression (psRDP) and BL Nf for overall population and psRDP tertiles representing fast (FP), intermediate (IP), and slow progressors (SP).

Results: No statistically significant differences in Nf were found between treatment groups (placebo vs tirasemtiv) at any time point; further analysis grouped all samples. At BL, pNfH and NfL did not differ by El Escorial category. There were no BL differences between shortest (<12 mo) and longest (>24 mo) symptom duration. BL Nf levels correlated with the psRDP (pNfH $r=0.29$, $p<0.001$; NfL $r=0.50$, $p<0.001$). For SP and IP, psRDP and NfL were not correlated, while for FP $r=0.43$, $p<0.0001$. Over 48 weeks, pNfH levels decreased versus BL, with the least squares mean difference (LSMD) first reaching significance at Wk 16 ($p=0.0003$); LSMD increased further with each 8-week interval ($p<0.0001$ from Wk 24 onward). NfL levels showed a less consistent pattern, but overall modestly decreased over time.

Discussion: VITALITY-ALS data is a large longitudinal study of Nf in an ALS clinical trial cohort. BL levels did not differ when considering ALS diagnostic certainty or symptom duration. There was only a moderate correlation between psRDP and BL Nf primarily driven by FP. The robust decline of pNfH over time compared with NfL levels may be related to plasma stability or differential release from neurons over time.

[1] Amyotroph Lateral Scler Frontotemporal Degener 2019;20:584-94.

294. Portable fixed dynamometry enables home-based, reliable assessment of muscle strength in patients with amyotrophic lateral sclerosis: a pilot study

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Objective: To determine the feasibility, reliability, and sensitivity of remotely monitoring muscle strength loss of knee extensors using a novel portable fixed dynamometer (PFD) in patients with amyotrophic lateral sclerosis (ALS).

Methods: We conducted a pilot study with a newly developed device to measure knee extension strength. Patients performed unsupervised PFD measurements, biweekly, for six months at home. We evaluated feasibility using adherence together with a device-specific questionnaire. Reliability was assessed by (1) comparing unsupervised and supervised measurements to identify systematic bias, and (2) comparing consecutive unsupervised measurements to determine test-retest reliability expressed as intraclass correlation coefficient (ICC) and standard error of measurement (SEM). Sensitivity to detect longitudinal change was described using linear mixed-effects models.

Results: We enrolled eighteen patients with ALS. Adherence was 86%, where all patients found that the device suitable to measure muscle strength at home; four patients (24%) found the measurements burdensome. The correlation between (un)supervised measurements was excellent (Pearson's r 0.97, 95%CI; 0.94 – 0.99) and no systematic bias was present (mean difference -0.13, 95%CI; -2.48 – 2.22, p = 0.91). Unsupervised measurements had excellent test-retest reliability with an average ICC of 0.97 (95%CI: 0.94 – 0.99) and SEM of 5.8% (95%CI: 4.8 – 7.0). Muscle strength declined monthly by 1.9 %predicted points (95%CI; -3.0 to -0.9, p = 0.001).

Conclusions: Using the PFD, it proved feasible to perform knee extension strength measurements at home which were reliable and sensitive for detecting muscle strength loss. Larger studies are warranted to compare the device with conventional outcomes.

295. Predicting Amyotrophic Lateral Sclerosis from Genotype Data Using Deep Neural Networks: Results from a 12-Strata Study

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Background

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons with an unknown genetic basis. Deep learning (DL) methods may help identify disease-associated variants that may be missed by traditional statistical approaches. However, many factors contribute to limit the use of DL for this aim including the challenge to account for population specific effect. As a result, current studies often use homogenous datasets form individual populations and an independent evaluation on international datasets comprising data from multiple populations is needed.

Method

We used a DL approach (1) based on Convolutional Neural Network (CNN) to predict the individuals who would develop ALS from microarray genotype data. We applied this method to 12 datasets from different European and North American countries with 23,503 cases and 12,587 controls from ProjectMine to test its applicability. We trained the CNN model using the genotype data from the cases and controls of each stratum, and evaluated its performance using accuracy, precision, recall, F1-score, and Area Under the Curve (AUC) metrics.

Results

Based on the results of our study, we found that the performance of CNN greatly varied across national datasets. CNN performed well in some populations, with the highest accuracy achieved in the Dutch population (93.1%) followed by the Italian population (93.0%). However, the approach performed poorly in other populations beyond sample size effects, with the lowest accuracy observed in Ireland (49.3%), and accuracies in other strata varying from 0.729 to 0.514. We also noted differences in precision, recall, and F1-score across the strata, suggesting that the performance of the model is influenced by population-specific genetic structures.

Discussion

Our findings suggest that the deep neural network approach is a promising tool for predicting the individuals that might develop ALS from genotype data. However, population-specific effects need to be considered when implementing this method. Further studies are needed to validate the effectiveness of this approach on larger and more diverse populations and should take into account the need for population-specific customization of the method.

Reference

Yin B, et al. Using the structure of genome data in the design of deep neural networks for predicting amyotrophic lateral sclerosis from genotype. Bioinformatics. 2019;35(14):i538-i547.

296. Prodromal phase of sporadic ALS: a patient and caregiver perspective

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Symptom onset of ALS is generally defined as onset of weakness or dysarthria. There is, however, less known about a prodromal phase of ALS. Mild motor impairment (MMI) is proposed to be part of the prodromal phase in genetic ALS, but it may not be necessarily limited to this concept. It is unknown when and how patients and their caregivers experience the onset of ALS.

The aim of this study was to investigate the patient and caregiver perspective on earliest signs of ALS, regardless of the current definition.

A cross-sectional, mixed methods study, combining a qualitative and quantitative approach, was conducted. An online survey on disease onset was sent to ALS patients registered at the UMC Motor Neuron Disease (MND) Biobank, an ongoing population-based study in the Netherlands, and their caregivers. Qualitative data was analyzed using Nvivo 12, emic and etic coding, thematic categorization and a thick description was established.

279 ALS patients and 150 caregivers participated. Preliminary results revealed main themes addressed by participants containing motor signs, subtle motor signs or sensory complaints. Motor signs included weakness, reported most frequently when compared to other topics. Additionally, cramps, fasciculations, speech problems, fine motor function (for example difficulty writing), and problems in relation to walking (altered walking pattern or a dragging leg) were mentioned in this category. Subtle motor signs were reported in relation to loss of control of limbs and balance disorder. Sensory disorders implied tingling and painful sensations mainly present in combination with weakness. Furthermore, reduced sport performance and feeling slowed down were reported as initial signs. Reported time of onset was mainly concordant with evident disease signs.

Prodromal symptoms known from other neurodegenerative diseases including, but not limited to, loss of smell, sleeping problems, obstipation and hallucinations were not reported.

Initial complaints of ALS predominantly imply motor signs, whereby evident

prodromal signs are not reported. Complete results of our analyses, including the timing of onset of symptoms and observations of the caregiver, will be presented.

297. Proximal Hirayama Disease

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A 15-year-old boy complained of proximal muscle weakness of the right arm that he first noted six months prior to his visit. Neurological examination revealed atrophy and weakness of the following muscles: deltoids, biceps, triceps and serratus anterior muscle on the right side, with scapular winging on that side. EMG study pointed to acute and chronic neurogenic signs in muscles innervated by the C5-C7 roots on the right. Cervical MRI revealed a forward displacement of the posterior cervical dural sac during neck flexion, causing compression of the cervical cord, and resulting in atrophic and ischemic changes in the anterior horn. Proximal muscle weakness and atrophy as presenting symptoms in Hirayama disease are extremely rare.

298. Quantitative evaluation of factors contributing to the results of RNS Test on ALS patients at initial visit.

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Background: To find out major factors contributing to the decremental response at 3Hz RNS test delivered to the accessory nerve and compare their respective contributions in amyotrophic lateral sclerosis(ALS) patients at the initial visit.

Methods: Clinical data involving 615 patients diagnosed as definite or probable ALS at Neurology Department of the First Medical Center, Chinese PLA General Hospital from June, 2016 to September, 2022 were collected to identify independent factors affecting decremental response by Spearman correlation and evaluate their respective contributions according to the results of multiple regression analysis.

Results: At the initial visit, the positivity rate of 3Hz RNS test delivered to the accessory nerve is 45.8%. It reaches 73.0% when motor unit potential(MUP) duration is larger than 12.9ms, and 70.8% when duration increment is over 32%. It's found that onset site, diagnostic delay, age at onset and MUP duration are independent factors contributing to the decremental response, where logarithmic superposition effect exists among the latter three ones. A 1% increase in onset age, diagnostic delay, or MUP duration, is associated with a 4.3%, 1.1% or 20.3% increase in amplitude decrement respectively. Besides, for patients with lower limb onset and bulbar onset, the expected changes in amplitude decrement decrease 4.2 and 6.1 percent respectively in comparison with patients with upper limb onset.

Conclusions: MUP duration has the greatest impact on decremental response, followed by onset age and diagnostic delay. Patients with upper limb onset has the highest risk of decrement response, followed by lower limb onset and bulbar onset.

299. Quinine for the treatments of muscle cramps in amyotrophic lateral sclerosis; a randomized, placebo controlled, double-blind cross-over trial

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BACKGROUND: Quinine has been used for decades for the treatment of muscle cramps, but it's use has been restricted by regularity authorities. We investigated the treatment effect of quinine on muscle cramps frequency and intensity in patients with amyotrophic lateral sclerosis (ALS).

METHODS: This was a randomized, double blind, placebo-controlled, crossover investigator-initiated trial. ALS patients with daily muscle cramps with a severity score ≥ 3/10 were randomized into two treatment arms starting with placebo or verum (250mg Quinine), respectively. A lead-in phase over 4 weeks was followed by 2 weeks of each treatment. After a washout phase of 4 weeks, the second 2-week treatment phase was followed by another washout phase of 4 weeks. Patients could continue with a one month open-label extension period (OLE). Primary outcome measures were frequency of diary-reported muscle cramps during day, night and subjective cramp intensity. Data were analysed using regression models.

RESULTS: Only 8 of intended 20 subjects could be recruited. No significant AE or SAE occurred. A statistically significant reduction of muscle cramp frequency during day (-47%, $p<0.03$) and intensity of cramps (-38.5%, $p<0.05$) was observed. No sequence- or period-effect was observed. Reduction of cramp intensity (-65.8%) and frequency (-72.9%, day) during the OLE phase was even higher compared to the verum phase only during daytime, but not significantly during night.

CONCLUSION: Quininesulfate is effective in reducing muscle cramp frequency and intensity in ALS patients. Inclusion criteria and prescribable access to the investigational product severely hampered recruitment into this study

300. Remote monitoring of accelerometry for amyotrophic lateral sclerosis detects differences in disease progression and survival

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Background: The considerable degree of heterogeneity among patients with amyotrophic lateral sclerosis (ALS) complicates the quantification of disease progression during clinical trials. Remote accelerometer-based outcomes may aid in capturing the functional benefits of promising therapeutic interventions in real-world settings. However, validation with key efficacy outcomes remains essential before implementation in these trials is possible.

Objectives: To determine the ability of remote accelerometry to detect differences in disease progression rate and survival time.

Methods: The data originated from two recent Dutch longitudinal cohort studies. In both cohorts, patients with ALS wore an accelerometer device (ActiGraph) for 7 days every 2 - 3 months. An accelerometer-based outcome (A1) was calculated based on movement against gravity aimed to reflect daily physical capability. Additionally, risk profile scores based on the ENCALS prediction model were used to describe disease progression rates. Statistical analyses were conducted within the joint-modeling framework.

Results: Between cohorts, patient demographics and clinical characteristics were similar and therefore combined (n = 99). Patients wore the ActiGraph for 1,995 days, for a total of 27,701 h (on average 13.9 h/day). Each patient was monitored for a median of 9.6 months with a maximum follow-up duration of 30 months. In total, 31 patients died. A1 significantly progressed over time ($p < 0.001$), as reflected by an average monthly decrease of 0.03 units. Furthermore, A1 progression had a significant interaction with risk profile scores ($p < 0.001$). After accounting for these scores, A1 was significantly associated with survival ($p < 0.001$). More specifically, a 0.50 unit decrease in A1 corresponded to a hazard ratio of 2.39 (95% CI 1.09 – 5.21).

Discussion: Accelerometry is an objective method to detect differences in disease progression and survival in real-world settings. Additionally, remote monitoring may reduce the need for in-clinic assessment, lower the burden of trial participation, and result in a more patient-centric clinical trial model.

301. Respiratory function conservation in ALS is potentially linked to modulation of the innate immune system

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Objective: Neuvivo reanalyzed clinical data from the first phase 2 study of NP001, performed biomarker studies, and in accordance with FDA guidance published in 2019 defined a subgroup of patients that responded clinically to NP001. The objective of the current post hoc study was to test whether regulation of the innate immune system with NP001 would be associated with effects on respiratory vital capacity (VC).

Background: Recent studies have confirmed that inflammation associated with ALS pathogenesis begins at the neuromuscular junction (NMJ) and involves innate immune system activated macrophages derived from blood. VC measurements define the function of the NMJ between the phrenic nerve and the diaphragm, potentially linking innate immune function with a measurable, survival associated clinical outcome.

Design/Methods: To understand the role of the innate immune response in progressive VC loss we evaluated data derived from files reported to the FDA on the 6-month placebo controlled double blind phase 2A study of NP001 in ALS patients within three years of symptom onset (NCT01281631). Plasma specimens stored at -80C were used to evaluate components of the humoral innate immune system from patients who received 2 mg/kg NP001 or placebo. C reactive protein (CRP) of ≥1.13 mg/L, a prespecified plasma measure of inflammation, was used to define patient groups. as high or low CRP. Only patient records from those < 65 years of age who completed the six-month study and had plasma available were included.

Results: ALS demographic baseline values were similar between treated and controls. High CRP patients showed no age associated loss of VC whereas low CRP patients showed an age dependent loss of VC function. High CRP NP001 treated patients showed a 64% slower rate of VC decline compared with placebo and those with low CRP who showed no VC response. Plasma levels of serum amyloid A (SAA) were elevated in high CRP patients consistent with ongoing innate immune system activation. Plasma TGFB1, a dominant regulator of nuclear factor kappa B (NF-kB) in high CRP treated patients was 95% higher than placebo at 6-months confirming the activation and release of this anti-inflammatory factor by the innate immune alpha 2 macroglobulin (A2M) system.

Conclusions: This report is the first to link a therapeutic approach for controlling VC loss in ALS patients with biomarker confirmed regulation of the innate immune system.

302. Results from first-in-human study of VRG50635, a PIKfyve inhibitor for treatment of ALS, in healthy adult volunteers

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Amyotrophic lateral sclerosis (ALS) is a disabling and fatal disorder characterized by progressive paralysis of voluntary muscles due to loss of motor neurons in the brain and spinal cord. Verge has identified a pathological deficiency in pathways from patients with sporadic and familial ALS. Within dysregulated pathways, the CONVERGE AI/machine learning platform identified PIKfyve/FIG4 as the top corrective drug target candidate. PIKfyve, a phosphoinositide kinase, has been implicated in regulating endolysosomal trafficking, exocytosis, and autophagy. Inhibiting PIKfyve improves motor neuron health and survival in preclinical ALS models (Shi 2018; Hung 2023). VRG50635 is a brain penetrant, orally administered small-molecule PIKfyve inhibitor under investigation for treating all forms of ALS.

The Phase 1 study of VRG50635 (EudraCT: 2022-002747-22) is a first-in-human, placebo-controlled study in healthy adults investigating the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics of VRG50635 in single (SAD) and multiple (MAD) ascending dose cohorts. Here we present preliminary blinded safety and PK data.

46 healthy subjects, randomized into five SAD cohorts, received VRG50635 at 60, 180, 540, and 1000 mg or placebo. A pilot food effect investigating the PK of VRG50635 (prodrug) and its active metabolite VRG50468 was completed in fasting (SAD cohorts 1, 2, and 3), high-fat meal (SAD cohort 3 crossover), or regular meal (SAD cohorts 4 and 5; MAD cohort 1) cohorts.

Preliminary blinded safety and tolerability data indicate that VRG50635 has been well tolerated. No SAEs or significant AEs have been reported. All AEs were low grade (97% G1), with treatment-emergent AEs observed in 39% of subjects. There have been no study withdrawals or discontinuations. No dose-related or clinically significant trends have been observed in vital signs, ECGs, physical examinations, or laboratory parameters.

Following administration of VRG50635, the prodrug concentrations were generally below the limit of quantification (1 ng/mL). PK of VRG50468 showed dose-proportional increases in Cmax and AUC. A positive food effect was observed with both high-fat and regular meals. A terminal half-life of approximately 37 hours supports once/day dosing and is consistent with increasing Cmax and AUC observed in repeated daily dosing in the MAD1 cohort. These data support the continued development of VRG50635 as a potential treatment for all forms of ALS via PIKfyve inhibition.

303. Retinal degeneration in Amyotrophic Lateral Sclerosis phenotypes – preliminary data from a longitudinal study.

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Retinal measurements are biomarker candidates for many neurological diseases, but their significance in Amyotrophic Lateral Sclerosis (ALS) remains unestablished. We investigate retinal degeneration in ALS and its different phenotypes, its value as a progression biomarker and its prognostic implications.

Methods: retinal Nerve Fiber Layer (RNFL) and Ganglion Cell Layer (GCL) are measured by Optical Coherence Tomography in 61 ALS patients and 12 healthy controls. Visual evoked potentials testing and cognitive assessment of ALS subjects are conducted concurrently, together with clinical examination, disease history collection, ALS-Functional Rating Scale (ALS-FRS) administration and blood collection for neurofilament (Nfl) levels determination. All tests are repeated after 12 months to detect progression. The latest available vital status information is used for analysis.

Results: when compared with controls, ALS subjects show lower mean GCL thickness in both eyes (right/left: 78,24/77,13 vs 83,5/83 µm – p 0,052/0,047) and global minimum GCL thickness (68,15 vs 78,17 µm – p
0,026). RNFL also shows thinning, without reaching statistical
significance (right/left: 89,39/89,57 vs 94,66/95,41 µm – p 0,116/0,084). Preliminary longitudinal analysis on 15 subjects reassessed after 12
months only shows left-eye GCL thinning (mean/minimum 78,67/71,73 vs 74,47/69,90 µm – p 0,014/0,077).

Conclusions: we detected GCL thinning transversally and longitudinally, confirming retinal degeneration as a neurodegeneration marker. Broader analysis, including additional follow-up data, will explore the relationships between such phenomenon and different motor and cognitive involvement patterns, progression rate and Nfl levels.

304. Role of traumatic injury in Amyotrophic Lateral Sclerosis: a "double-hit" pathogenic model of neurodegeneration

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder that mainly affects both upper and lower motor neurons. Although ALS pathophysiology involves various underlying genetic mutations (such as SOD1, C9orf72, FUS, and TDP43), many patients suffer from the sporadic form of the disease. Interestingly, previous studies have suggested that environmental factors such as smoking, alcohol consumption, heavy metals, and traumatic injury are implicated in the pathogenesis of ALS. Contact sports are associated with head and spine trauma, which causes a significantly higher risk of developing ALS. This suggests that repeated traumatic events may play an active role in ALS pathogenesis. However, the molecular mechanisms through which repeated traumatic events lead to ALS have still to be clarified. Therefore, we aimed to investigate whether physical trauma might represent a pathogenic trigger leading to the anticipation of ALS manifestation in genetically vulnerable individuals. To investigate this, we differentiated three-dimensional cerebral and spinal organoids from human induced pluripotent stem cells (hiPSCs) from healthy controls, C9orf72 patients and asymptomatic C9orf72 carriers. After three months of differentiation, we simulated traumatic injury in hiPSC-derived organoids placed into 3D-printed bioscaffolds using the weight drop method. We then performed protein and transcriptional analyses to investigate the effects of traumatic injury in C9orf72-carrier organoids to evaluate whether external insults might trigger the appearance of canonical ALS hallmarks such as aggregates accumulation, cell death and expression of pro-inflammatory genes, thus resulting in a pathogenic "second-hit". Our approach aims at clarifying how repeated trauma might actively contribute to ALS pathogenesis.

305. Safety and Activity of Anti-CD14 Antibody IC14 (atibuclimab) in ALS: Experience with an Expanded Access Protocol

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Introduction: Expanded access protocols (EAPs) provide access to investigational products to patients who do not qualify for clinical trials. IC14 is a monoclonal anti-CD14 antibody that may target T-regulatory (T-reg) cell function. We administered IC14 to 21 individuals with ALS via an EAP and documented target engagement, safety, and disease endpoints.

Methods: Participants received intravenous IC14 at a dose of 8 mg/kg every 14 days. Two dose escalation phases increased the dose from 8 mg/kg to 20 mg/kg. Once participants reached the highest dose, infusion frequency was reduced to 21-day intervals. All participants were ineligible for clinical trials. Participants unable to travel to the site due to the COVID-19 or disease progression were transitioned to infusions at-home/locally. Blood samples for hematology, chemistry, and coagulation were collected to monitor safety. The Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised was administered every 4 to 6 weeks. Respiratory function was measured through Slow Vital Capacity tests. Whole blood and serum were collected to determine monocyte CD14 receptor occupancy (RO) and anti-drug antibodies (ADA). T-reg cell functional assays were performed starting with the fifth participant.

Results: Participants received IC14 for up to 143 weeks. Treatment-emergent adverse events were mild and self-limited. There were 27 serious adverse events (SAEs) which were unlikely or unrelated to IC14. One SAE of an allergic reaction was related to IC14 and expected. Six participants died due to disease progression. 32.8% of all infusions were administered locally or at home. At 8 mg/kg, sustaining ≥80% RO required dosing at 14-day intervals. RO assays performed throughout the program necessitated a dose escalation to sustain ≥95% RO levels. RO levels achieved with 20 mg/kg allowed for decreased visit frequency to every 21 days which lessened visit burden. Longitudinal T-regs isolated after IC14 administration showed enhanced T-reg suppression of autologous CD4 T cell proliferation.

Conclusion: IC14 administration was safe, well tolerated, with no significant changes in laboratory tests, and no unexpected drug-related SAEs in this EAP. Measuring RO guided dose and dosing frequency. Preliminary data suggest IC14 enhances T-reg activity. Data collected during the EAP will not replace data generated in clinical trials. However, EAPs can provide supplemental safety information from a broader patient population.

306. Safety, Pharmacokinetics, and Pharmacodynamics of the Selective Glucocorticoid Receptor Modulator Dazucorilant in Healthy Volunteers

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Introduction: Hypothalamic-pituitary-adrenal axis dysregulation plays a role in amyotrophic lateral sclerosis (ALS). Dazucorilant (DAZU, CORT113176) is a selective glucocorticoid receptor modulator in development for the treatment of ALS. In Wobbler mice, a model of sporadic ALS, DAZU reduced forepaw atrophy, improved performance in the rotarod test, and inhibited neurodegeneration and neuroinflammation. Here, we describe the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of orally administered DAZU capsules in healthy volunteers.

Methods: A phase 1, first-in-human, adaptive dose, double-blind, placebo-controlled study (NCT04249323, EudraCT 2019-004258-27) tested single (50–1000 mg) and multiple (100–300 mg QD for 14 days) DAZU doses with or without food in 110 healthy male and female volunteers. Safety, tolerability, PK, and PD were assessed. In a phase 1, randomized, partially double-blind, placebo-controlled study in 16 healthy male volunteers, DAZU concentrations in the cerebrospinal fluid (CSF) were assessed (NCT04994743, EudraCT 2021-002456-36).

Results: In the first-in-human study, a greater than dose-proportional increase in DAZU exposures and a positive food effect were noted. Multiple dosing led to an approximately 2-fold accumulation in plasma; steady state exposures were achieved in approximately 7 days. As expected, prednisone 25 mg decreased eosinophils, lymphocytes, and osteocalcin, and increased neutrophils. There was a statistically significant difference in area under the change from baseline curve from 0 to 24 h for eosinophils, lymphocytes, and osteocalcin with prednisone + DAZU 450 mg, suggesting that DAZU ameliorates the effect of prednisone on these biomarkers. Single doses of DAZU up to 1000 mg fasted and 900 mg fed were considered safe and well tolerated. Multiple doses up to 300 mg QD were considered safe; multiple doses up to 200 mg QD were generally well tolerated. No serious or severe treatment-emergent adverse events (AEs) were reported; the most common AEs were gastrointestinal, nervous system, musculoskeletal and connective tissue disorders. DAZU distribution to the CSF was observed, confirming brain penetration.

Conclusions: The presented studies established the PK, safety, tolerability, and pharmacological effect of DAZU in healthy volunteers. A phase 2 study (DAZALS, NCT05407324, EudraCT 2021-005611-31) is ongoing to assess whether DAZU can benefit patients with ALS by slowing functional loss.

307. Sex-related differences in Amyotrophic Lateral Sclerosis: a brain 2-[18F]FDG-PET study

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Introduction. The neuroanatomical correlates of sex differences in Amyotrophic Lateral Sclerosis (ALS) have been studied in animal models and in human neuropathological series, but they still remain unclear. In this study, using a robust 2-[18F]FDG-PET dataset of ALS patients and healthy controls (HC), we focused on the metabolic brain differences related to sex, adjusting for factors contributing to phenotypic heterogeneity.

Methods. We collected two equal-sized groups of male (m-ALS) and female ALS (f-ALS) patients (n=130 each), who underwent 2- [18F]FDG- PET at diagnosis. They were matched for site of onset (bulbar/spinal), cognitive status (normal/impaired), and King's stage. We included two gropus of 84 male (m-HC) and 84 female (f-HC) age-matched HC. We compared m-ALS and f-ALS including age as covariate on one hand, and m-HC and f-HC on the other hand, employing the two-sample t-test model of SPM12. To further characterize the metabolic differences between m-ALS and f-ALS, a differential network analysis was performed. Starting from each patient, 94 brain ROIs and metaROIs were extracted, along with their respective (normalized) metabolic levels. By building two correlation networks we assessed the difference in terms of connectivity between m-ALS and f-ALS.

Results. F-ALS showed clusters of relative hypermetabolism including bilateral medial frontal, parietal, and occipital cortices, and left temporal cortex, compared to m-ALS. No significant difference emerged between m-HC and f-HC. In node-wise comparison between m-ALS and f-ALS, 2 metaROIs showed significantly higher connectivity in f-ALS compared to m-ALS (right mid cingulate cortex and left superior and medial frontal gyrus).

Conclusions. Sex resulted a major determinant of brain metabolism in ALS patients and underlay differences in brain connectivity. Sex should be considered an essential factor in the evaluation of results in clinical trials, since sex-related brain metabolic differences might be associated with a heterogeneity in the response to treatments.

308. Social Cognition and Executive functions in Amyotrophic Lateral Sclerosis: a population-based study.

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Background: Impairment in Social Cognition (SC) has been reported in Amyotrophic Lateral Sclerosis (ALS) since the early stages of disease and SC deficits have been included in the revised Strong criteria. However, cognitive determinants of SC subdomains are largely unknown, and, in particular, the functional relationship with executive functions (EF) is a matter of debate. The aim of the present study was to explore the correlation between SC abilities with EF in ALS patients, and to evaluate the impact of SC assessment on the cognitive-behavioural classification in this population of patients.

Methods: We enrolled 102 consecutive patients and 50 healthy controls attending the Turin ALS Center between February 2019 and December 2022. All patients and controls underwent a neuropsychological battery assessing all fundamental cognitive domains, included SC, through the Ekman 60-Faces test (EK-60F), the Reading the Mind in the Eyes Test-36 faces full version (RMET-36), and the Story-Based Empathy Task (SET).

Results: EK-60 F test did not show any significant correlation with Executive functions tests. RMET-36 showed an overall moderate significant correlation with the other cognitive tests, and a significant specific correlation with Wisconsin Card Sorting Test (WCST) (p 0.037). SET-GS showed an overall moderate significant correlation with the other cognitive tests, and a significant correlation trend with Trial Making Test (TMT) B-A (p 0.049). Taking into account the SC assessment, out of 61 ALS-CN patients, 5 (8.1%) were reclassified as ALSci, and one (1.4%) out of the 7 ALSbi patients, was reclassified as ALScbi. These reclassified patients were mostly male (5 male and 1 female), with spinal onset (4 with spinal-onset and 2 with bulbar-onset), and 1 of them carried a genetic mutation in the c9orf72 gene.

Conclusions: Our study confirmed the higher sensitivity of the revised criteria for detecting early cognitive signs in ALS patients, and highlights the substantial impact of SC on cognitive-behavioural categorization of ALS patients. For these reasons, SC assessment can help care-givers to develop awareness about the subtle cognitive-behavioural alterations that can arise since the early stages of the disease, help clinicians to timely discuss and manage patients' therapeutic choices, and can also have significant prognostic implications.

309. SOD1-ALS: patient journey in the tofersen early access program

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Currently, in the vast majority of patients with amyotrophic lateral sclerosis (ALS) no genetic cause can be detected. However, approximately 2 % of all cases are linked to mutations in the superoxid dismutase 1 (SOD1) gene. With tofersen, the first intrathecally administered antisense oligonucleotide therapy for ALS has been developed to reduce SOD1 protein synthesis. Although the VALOR-trial did not reveal significant clinical effects on the revised ALS functional rating scale (ALSFRS-R) as the primary study endpoint over 28 weeks, tofersen led to a strong reduction of SOD1 protein in cerebrospinal fluid (CSF) and neurofilament light chain (NfL) concentrations in plasma. An open-label extension phase is ongoing to further evaluate potential drug effects. In 2021, Biogen has initiated an early access program for treatment of patients with SOD1-ALS.

This is to present the individual treatment course of a 40-year-old female patient diagnosed with spinal-onset ALS and confirmed c-358-10T>G SOD1 variant. After given informed consent and screening eligibility criteria, the early access program has started on 18 October 2022 by first intrathecal application of tofersen.

Data on clinical characteristics, laboratory values as well as adverse events and feasibility during treatment were collected. Clinically, motor functions and physical disability remained largely stable (ALSFRS-R baseline score: 34/48 points, baseline slope: 0.93 points/month; last assessed ALSFRS-R score: 33/48 points, last assessed slope: 0.79 points/month). In line with findings from the VALOR-trial, NfL concentrations in CSF decreased during treatment (NfL baseline concentration: 8202.7 ng/l; last assessed NfL concentration: 3693.0 ng/l). Serum creatine kinase (sCK) as a marker of muscle destruction decreased likewise (baseline sCK concentration: 336.0 IU/l; last assessed sCK concentration: 157.2 IU/l).

Serious adverse events have not occurred to this day, and intrathecal administration has been well tolerated. Only pseudoradicular syndrome and temporary mild pleocytosis were reported after the 1st and 5th administration, respectively. Due to these favorable circumstances and individual's treatment satisfaction, the patient is willing to further participate in the access program.

310. Stepwise functional brain architecture from disease epicenter correlates with atrophy in progressive supranuclear palsy

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Introduction. MRI connectomics tests the model of network-based spread of pathological aggregates in neurodegeneration. Stepwise functional connectivity (SFC) is a graph-theory-based neuroimaging method detecting functional couplings of a selected region, at increasing link-step topological distances. SFC was applied to test if topological stepwise architecture propagating from disease epicenter would shape patterns of grey matter (GM) atrophy in progressive supranuclear palsy (PSP).

Methods. Twenty-eight patients with PSP and 50 healthy controls underwent 3D-T1 and resting-state functional MRI sequences. GM was parcellated into 90 regions. Correlations between SFC architecture in controls and atrophy patterns in patients were tested. Disease epicenter was identified as the peak of atrophy in an independent cohort of 13 cases with post mortem PSP pathology, and used as seed region for SFC analysis.

Results. Left midbrain tegmentum was identified as disease epicenter. Compared with controls, PSP patients showed atrophy of subcortical GM (thalami and caudate nuclei), and frontal, parietal and cerebellar cortex. For each region, a strong correlation was found between average link-step distance from the left midbrain in controls and mean normalized GM volume in PSP patients ($r=0.37$, $p<0.001$).

Conclusion. The brain architectural topology, as described by SFC propagating from disease epicenter, shapes the pattern of atrophic changes in PSP. Stepwise analysis holds the promise to be used to model disease progression in future longitudinal studies.

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311. Study design for an open-label, randomized, 2-arm trial of sotuletinib (BLZ945) in people with amyotrophic lateral sclerosis

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Background: Microglial activation is an important contributing factor to the pathophysiology of amyotrophic lateral sclerosis (ALS). Microglia require signals from colony stimulating factor-1 receptor (CSF-1R) for survival and proliferation. Therefore, selective inhibition of CSF-1R using sotuletinib (BLZ945) to deplete microglia may represent a potential therapeutic strategy for disease modification in ALS. Sotuletinib is a highly potent and selective inhibitor of CSF-1R under investigation for ALS.

Objective: This open-label, randomized, 2-arm trial (cohort 5) is designed to evaluate safety, microglia reduction as measured by translocator protein (TSPO) positron emission tomography (PET substudy at qualified PET centers), and changes in neuroinflammatory and neurodegenerative biomarkers in blood and cerebrospinal fluid (CSF substudy) after administration of repeated cycles of oral sotuletinib over 12 weeks using two different dosing regimens.

Methods: This phase 2 study (ClinicalTrials.gov Identifier: NCT04066244) will be conducted at multiple sites in USA, Finland, Sweden, and Australia. The study consists of completed cohorts 1–4 with single group design and an ongoing cohort 5 with a randomized, parallel design. Study population comprises of male and female participants aged ≥18 years at screening diagnosed with familial or sporadic ALS. Participants from cohorts 1 to 4 will be allowed to participate in cohort 5 if they meet eligibility criteria. Participants in cohort 5 will be randomized (1:1) across two dosing regimens: (arm 1) sotuletinib administered once daily for the first 4 days of a 2-week period in repeated cycles (i.e., 4 days on treatment and 10 days off [4/10]) or (arm 2) sotuletinib administered once a week. Cohort 5 will include screening of up to 6 weeks, a 12-week open-label treatment, followed by a 4-week safety follow-up (plus 8 weeks for recovery in participants with findings). Participants who consent and are eligible may continue for an additional 24-week extended treatment on the same regimen and will be followed up for 1 year.

Results: Enrollment into cohort 5 was initiated in January 2023. There were four participants enrolled and randomized in cohort 5 at the time of abstract submission.

Conclusion: Cohort 5 study is expected to provide evidence of safety and evaluate two planned sotuletinib dosing regimens to inform future sotuletinib clinical studies in people with ALS.

312. Systematic review of the methodological quality of clinical trials in Amyotrophic Lateral Sclerosis

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Several clinical trials have been performed in MND/ALS but no active substance effective on disease progression have been found so far, with the exception of a minor effect on survival with riluzole and Edaravone.

We conducted a systematic review to evaluate the methodological quality of clinical trials performed on ALS. This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA). 3 pairs of independent evaluators performed data extraction. 3 meeting were performed in order to discuss any issue or discrepancy during data extraction activities. Rob 2 for parallel and cross over study were used to detect risk of bias.

266 manuscripts were included in our systematic review.

52% of evaluated manuscripts described preclinical study evaluation, among these, a positive effect was reported for only 55% of the studies. Toxicity evaluation was described only in 28% of manuscripts.

66% of trials were conducted with a parallel arm design, while 12% were cross over studies; 74% were randomized, while in 7% historical controls were used for comparison. 67% of trials were double blind, among these, allocation concealment was described in 30%. An adaptive design was reported in 3% of studies.

According with inclusion criteria, subjects could be enrolled with FVC<50 in 10% of trials, SVC< 50% in 4% and 85% did not provide a minimun value for respiratory capacity at inclusion. Disease duration was <6 months in 2 studies, <36 months in 18, < 60 months in 7, while in 45 a disease duration was not described or was not considered in the inclusion criteria.

In 17% of studies the included subjects were not similar to those observed in the clinical practice, and in 10% the enrolled subjects were not similar between treatment arms.

Drop out rate was ≥20% in 29% of trials, while it was not reported in 9%. A positive clinical efficacy of the active principle under study was detected in 38% of studies.

The methodological quality of the included studies was highly variable, and many of them could be at high risk of bias. Possible shortcomings in trial methodology could have affected the study results.

Future trials should be performed with adequate study methodology and design, as well as a good results reporting, following the actual guidelines. This will ensure that the obtained results will provide useful information for the ALS community. This has not always been the case in the past.

313. Testing Spinal Motor Neuron Function Using Vibration-Evoked Persistent Inward Currents: A Preliminary Study

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Background: Whether motor neurons (MNs) generate an action potential depends on the summed activity of several sources of descending and afferent ionotropic inputs. Motor neuron firing patterns are modulated by both inhibitory and excitatory inputs . These inputs are powerfully influenced by the neuromodulatory system. Persistent sodium and calcium inward currents (PICs) amplify and prolong synaptic input and are therefore relevant for assessing altered function in ALS. Vibration applied to the distal tendon elicits phase-locked firing of Ia afferents, thus activating the myotatic pathway and PIC-mediated muscle contraction. We measured the reflexive torque in response to vibration which is highly dependent on the monoaminergic regulation of intrinsic membrane potential.

Methods: Focal vibration at 25Hz (sham), 60Hz, 70Hz, and 80Hz was applied to the flexor carpi radialis distal tendon in five healthy controls. Vibration was preloaded on the tendon at a baseline of 1.7 Newton and lasted for 8 seconds per trial. Tendon collision force, vibration amplitude, and reflexive torque generated by the wrist was recorded during each frequency as the reflex response is known to be strongly modified by these parameters. Wrist flexion torque, peak torque and rise slope were calculated for each frequency.

Results: group averaged reflex torques revealed that low frequency (25Hz sham) high amplitude displacements increases the baseline collision force but does not evoke reflexive torque. Very low amplitude, high frequency (80Hz) vibration does not significantly differ to sham stimulation in reflexive torque generated. High amplitude and high frequency conditions 60Hz ($P<0.01$) and 70Hz ($p<0.05$) are significantly more effective for inducing the reflex compared to 25Hz and 80Hz.

These results indicate that vibration at higher amplitude and frequency (60Hz and 70 Hz) evokes a stronger reflexive torque compared to very low amplitude (80Hz) or frequencies that are below the known frequency range to stimulate Ia afferents (25Hz). The interaction of frequency and amplitude are essential parameters for reliably eliciting reflexive torque associated with the action of PICs in spinal motor neurones. Future work will compare results of younger and age-matched controls to ALS which may reveal vibration as a valuable tool to assess degeneration in ALS.

314. The deltoid muscle – part of the monosynaptic pattern of pareses in ALS

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The pattern of pareses in ALS is consistent with the physiology of the human corticospinal tract, mirroring the phylogenetically young monosynaptic connection of the motor cortex and anterior horn cells (Ludolph et al., 2020).

We previously did not involve the deltoid muscle into these arguments; the deltoid is affected frequently and early by the disease process. In this study we prospectively evaluated the degree of paresis of this muscle by measuring the BMRC score of the deltoid and compared it with the neighbouring biceps and triceps brachii in 72 patients (432 muscles).

We could show that the extent of involvement of the deltoid in the disease process resembles the severe pareses of the biceps ($p = 1.00$), but the deltoid was statistically significantly weaker than the triceps brachii ($p < 0.01$). This pattern is consistent with functional data of corticospinal monosynaptic connectivity of these three muscles (Colebatch et al., 1990). The authors of this electrophysiological study concluded that "the strength of the connections to the deltoid .... is similar to that of an intrinsic muscle of the hand....".

Therefore, we conclude that the major involvement of the deltoid in the disease process in ALS is consistent with a cortical pattern of pareses (Braak et al., 2013).

315. The Edinburgh Brain Bank – a global leader in discovery science and reverse translation in motor neuron disease

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ntroduction: Studies of neuropathological tissue from people with MND (pwMND), linked to highly curated clinical phenotypic data, are crucial in advancing understanding of pathogenesis, reverse translation, and biomarker discovery. The Edinburgh Brain Bank (EBB) is a global leader in neurodegenerative disease research with brain tissue samples acquired since 2005. We report on the trend in brain and spinal cord donations made from pwMND and highlight the breadth of bench-to-bedside discovery projects supported.

Background: Scotland has evolved as a global leader in MND clinical care and discovery research over the last decade, including the following:

1) A doubling of nurse specialists (currently 1 specialist: 44 pwMND)

2) Overhaul of the Scottish MND Register (CARE-MND): 99% ascertainment and highly annotated longitudinal phenotyping with 2700 registrations since 2015

3) Delivery of the UK's largest definitive multi-arm multi-stage Phase 3 trial for MND (MND SMART) since 2020

4) A rich culture of consenting for brain and spinal cord donation.

Methods: We conducted a retrospective analysis of EBB data from 2015–2023 with an analysis of MND tissue donation per year and summarised the range of scientific outputs supported by this resource.

Results: The brain bank has collected a total of 832 tissue samples from healthy controls and various neurological conditions, including MND (159 since 2007). From 2007 to 2015, the MND tissue donation ranged from 0 to 13 per year (mean 4.2). There has been a significant rise since, with annual donations ranging from 8 to 19 cases per year from 2015–2019 (mean 12.8), and 14 to 23 cases per year from 2020–2022. Discovery research supported by the EBB includes the identification of novel molecular targets in TDP-43 pathology, genomic studies, neuropathological characterization of polar clinical phenotypes using novel techniques such as spatial transcriptomics, and reverse translational projects including biomarker discovery.

Conclusion: Allied to a high level of clinical phenotyping, the EBB is a unique resource promoting interdisciplinary discovery science in MND. Targeted changes to patient engagement across Scotland, including both data-driven research and bioresource donations, have led to increased awareness of brain tissue donation for MND. The Scottish MND community is hugely committed to research, and through better engagement, the number of MND tissue donations per year has continued to rise.

316. The Effect of the Fat Content of Food on the Pharmacokinetics and Safety of Utreloxastat Oral Solution Formulation in Healthy Volunteers

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Background: Utreloxastat is an orally bioavailable inhibitor of 15-lipoxygenase that reduces oxidative stress and spares reduced glutathione. It is currently in development for the treatment of neurological diseases characterized by high levels of oxidative stress and mitochondrial pathology, including amyotrophic lateral sclerosis.

Aim: The aim of the present study was to (1) investigate the effect of food on the pharmacokinetic (PK) profile of utreloxastat, and (2) evaluate the safety of an oral solution formulation in healthy volunteers.

Methods: Fifteen healthy volunteers were enrolled in this randomized, open-label, single-dose, 3-period, 6-sequence, crossover study. Subjects were randomized into 1 of 6 sequences consisting of 3 4-day treatment periods, which determined if they were under a fasted or fed (low-fat or high-fat) state upon dosing with 250-mg of utreloxastat oral solution. Treatment periods were separated by a 4-day washout period. The primary PK endpoints evaluated were AUC0-t, AUC0-inf, Cmax, and Tmax.

Results: Blood samples collected during the treatment periods were analyzed for PK parameters, with the fed and fasted-state treatments used as test and reference, respectively. Administration of utreloxastat under the low-fat fed state resulted in geometric mean ratios of 3.518 and 2.641 for Cmax and AUC0-inf, respectively. Similarly, treatment under the high-fat fed state resulted in geometric mean ratios of 2.806 and 3.356 for Cmax and AUC0-inf, respectively. The median Tmax was 1.00, 2.01, and 3.02 hours under the fasted, low- and high-fat fed states, respectively. A total of 10 adverse events (AEs) were reported by 6 subjects during the study, of which 9 were considered treatment-emergent AEs (TEAE). All TEAEs were mild or moderate in severity.

Conclusions: Overall, the PK profiles of utreloxastat were comparable between the low- and high-fat fed states. When comparing the fed and fasted states, the mean PK profiles were approximately 3-fold higher, demonstrating that administration of utreloxastat with food increased systemic exposure. Safety evaluations revealed that food had no effect on the safety and tolerability of a single oral dose of utreloxastat. The drug was well tolerated with no clinically meaningful safety observations, and no deaths or serious adverse events reported.

317. The Impact of Non-Motor Symptoms in People with Motor Neuron Disease

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Background: In addition to changes in physical functioning, people with motor neuron disease (MND) often experience non-motor symptoms (NMS). These may occur secondary to, or distinct from, motor degeneration, and are becoming more widely acknowledged as prevalent and impactful features of the condition. The presence of NMS may significantly reduce quality of life despite and are often under-recognised and evaluated in clinical practice and trial outcomes.

Aim: To explore how frequently people with MND report symptoms of low mood, anxiety, cognitive impairment, behavioural change, fatigue, pain, disturbed sleep, problematic saliva, sexual dysfunction and gastrointestinal issues occurring.

Methods: People registered on CARE-MND platform (Clinical, Audit, Research and Evaluation, the Scottish MND Register), were invited to complete a bespoke questionnaire on NMS and a self-reported Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS(R)). The questionnaire involved a pre-defined list of 11 potential NMS, with the opportunity to list any additional symptoms occurring. This was supplemented by CARE-MND clinical data.

Results: 99% of participants (n = 120) experienced at least one NMS, with 72% (n = 120) reporting five or more. The NMS most often reported were pain and fatigue (reported by 76% of participants respectively). The symptoms reported to be most impactful were pain and problematic saliva (51% respectively). Lower ALSFRS(R) scores, older age at onset and being a long survivor (diagnosed over 7 years ago) were significantly associated with reporting more NMS. 73% of respondents were happy with the frequency that NMS were discussed in clinical care. 80% of participants indicated they believe NMS are important to include in trials, independent of their personal experience of these symptoms. The preferred method of assessment for NMS was completing questionnaires, at home.

Conclusions: The majority of people with MND report NMS and these frequently co-occur. Pain, fatigue, gastrointestinal issues, sleep, mood, anxiety, problematic saliva, apathy, emotional lability, cognitive impairment and sexual dysfunction are prevalent. Older individuals, with worse physical function and those who were long survivors were more likely to report more NMS. Where reported, these symptoms are frequent, impactful and a priority for people with MND in clinical care and trial design.

318. The Inclusion of Lytico-Bodig Biospecimens into the United States National ALS Biorepository

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Objective: To incorporate additional high-quality biospecimens, referred to as Guamanian ALS or Lytico-Bodig, into the National ALS Biorepository.

Background: Lytico-Bodig disease refers to the striking combined increased incidence of amyotrophic lateral sclerosis (ALS, “Lytico”) and Parkinsonism-Dementia Complex (PDC, “Bodig”) among the Chamorros on the island of Guam, first recognized in the 1940s and 50s. Frequently, ALS, parkinsonism, and dementia occurred in the same individuals and families. Over the latter half of the 20th century, the incidence of Lytico-Bodig decreased steadily, thus it represents a prototype disorder for examining the intersection of environment and genetics in motor neuron disease, and the factors influencing the overlapping clinical syndromes and pathologies of parkinsonism, dementia, and ALS. The Center of Guam collected autopsy tissues and detailed clinical and pathological records from 400-500 decedents who died from Lytico-Bodig, standard ALS, and non-neurological control decedents. Serum and cerebrospinal fluid (CSF) biofluid samples were also collected from a selection of this this cohort. These biosamples and the associated clinical and pathologic data have recently been curated at Binghamton University. In 2022, researchers approached the National ALS Registry, to incorporate this valuable resource into the National ALS Biorepository, in order to ensure that the tissue, biofluids, and associated data are responsibly curated and made broadly available to researchers.

Results: The Registry, along with researchers from the Temple University ALS Postmortem Core, undertook an examination and analyses of the Guam biospecimens and corresponding records. The identity of all samples and inventories were verified and cataloged according to our existing hierarchical anatomical nomenclature. Standardized and de-identified clinical and pathological data elements have been extracted and harmonized with our existing data elements. Several projects utilizing these resources are underway, including biochemical, quality control, pathological, and genomic analyses. The results from these analyses will be made broadly available and linked back to the samples themselves.

Discussion: There are still many unknowns about ALS, specifically the etiologies and risk factors. Integrating the Lytico-Bodig biospecimens and data in the National ALS Biorepository provides opportunity to better understand ALS risk factors and disease pathology, wh

319. THE INTEGRATED STRESS RESPONSE IS MODULATED BY EIF2B AGONIST DNL343: RESULTS FROM PHASE 1 HEALTHY SUBJECT AND PHASE 1B ALS PATIENT STUDIES

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Objective: DNL343 is being investigated as a potential therapeutic agent for ALS.

Background: ALS is a fatal neurodegenerative disease with TDP-43 inclusion pathology in 95% of patients. Chronic activation of the integrated stress response (ISR) may contribute to ALS by blocking translation, altering RNA and endosomal trafficking, and increasing formation of TDP-43-containing stress granules. DNL343 is a small molecule that activates key ISR regulator, eIF2B, which inhibits ISR stress granule formation in cellular models and promotes neuroprotection in animal models.

Methods: The safety, pharmacokinetics (PK) and pharmacodynamics (PD) of DNL343 were evaluated in a Phase 1 randomized, placebo-controlled trial (RCT) in ninety-five healthy participants (n=48 single ascending dose, n=47 multiple ascending dose [MAD]; NCT04268784) and a 28-day Phase 1b double-blind (DB) RCT in twenty-nine ALS participants (2 doses tested; NCT05006352), with an ongoing 18-month OLE. ISR inhibition was evaluated by measuring CHAC1 gene expression and ATF4 protein in stimulated peripheral blood mononuclear cells.

Results: In the Phase 1 study, DNL343 was generally safe and well-tolerated, showed extensive CSF distribution and a long half-life (38-46 hours) supporting once-daily dosing, and showed potent ISR biomarker responses in all MAD cohorts. Similarly, in the Phase 1b DB study, DNL343 was generally safe and well-tolerated with no serious adverse events. One discontinuation occurred due to rash. DNL343 plasma concentrations were dose-dependent and had similar concentrations in CSF as in plasma with a ratio of 1.2. DNL343 robustly attenuated two ISR biomarkers (CHAC1 [56-85%] and ATF4 [73-79%]) in the DNL343 treated cohorts.

Conclusion: DNL343 was generally safe and well-tolerated at doses that demonstrate robust inhibition of ISR through CHAC1 and ATF4 inhibition. The Phase 1 and Phase 1b pharmacokinetic profiles support once-daily oral dosing and along with preclinical in vivo data, are consistent with extensive CNS distribution. These data support further development of DNL343 as a novel investigational therapeutic for the treatment of ALS.

320. The landscape of neurophysiological outcome measures in ALS interventional trials: a systematic review

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Objective:

We collated all interventional clinical trials in amyotrophic lateral sclerosis (ALS), which utilised at least one neurophysiological technique as a primary or secondary outcome measure. By identifying the strengths and limitations of these studies, we aim to guide study design in future trials.

Methods:

We conducted and reported this systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Eight databases were searched from inception. In total, 703 studies were retrieved for screening and eligibility assessment.

Results:

Dating back to 1986, 32 eligible interventional clinical trials were identified, recruiting a median of 30 patients per completed trial. The most widely employed neurophysiological techniques were electromyography, motor unit number estimation (including motor unit number index), neurophysiological index and transcranial magnetic stimulation (including resting motor threshold and short-interval intracortical inhibition). Almost 40% of trials reported a positive outcome with respect to at least one neurophysiological measure. The interventions targeted either ion channels, immune mechanisms or neuronal metabolic pathways.

Conclusions:

Neurophysiology offers many promising biomarkers that can be utilised as outcome measures in interventional clinical trials in ALS. When selecting the most appropriate technique, key considerations include methodological standardisation, target engagement and logistical burden.

Significance:

Future trial design in ALS would benefit from a standardised, updated and easily accessible repository of neurophysiological outcome measures.

Reference:

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321. The perception of unpleasant sensations of patients with amyotrophic lateral sclerosis during procedures required by clinical trials (PESALS)

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Introduction: There is currently no cure for ALS and few treatments are available, however there are many new experimental therapies under development that are showing promise for the treatment of ALS. The research protocols can include diagnostic and/or therapeutic procedures, which the patient may have already undergone and for which he is aware that he will experience annoying and/or painful sensations. These sensations could compromise participation, adherence to the pathway envisaged by the clinical trial and the continuation of the same.

Methods: The study uses a descriptive phenomenological approach by Sundler et al. according to which the researcher must be open to the meaning of the experiences lived by the interviewee and assume a position of careful observer being able to question the understanding of the data, through a self-reflective attitude that allows him/her to become aware of the his prejudice. Based on the experience of the researchers and the recommendations proposed by Sandelowski, a total of 20 interviews are estimated in order to reach the theoretical saturation per category of reference. Data collection will be carried out through in-depth semi-structured interviews recorded, subject to patient consent (13 open-ended questions after the execution of the procedures).

Objective: The objective of the study is to investigate the perception of unpleasant sensations of ALS patients participating in clinical trials which contain procedures, including the different methods of administration of the experimental drug.

Specific aims: Understanding the experience and opinions of the perception of unpleasant sensations during the procedures foreseen within clinical trials of patients with ALS will allow to identify future personalized strategies with the possibility of carrying out interventions aimed at reducing and increasing tolerance to annoying procedures and/ or painful. Furthermore, this could improve adherence to the clinical trial pathway, through the reduction of anxiety states generated by annoying/painful procedures.

Implications for future research: This can represent an approach to improve the quality of care and the taking charge of patients' health conditions by the nurse and the medical team, implementing strategies for multidisciplinary care and assistance; in order to guarantee a better quality of ALS patients' life.

322. The relationship between quality of life and cognitive or behavioural impairment in motor neuron disease: a systematic review

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Background: Motor neuron disease (MND), progressively impacts physical function and self-perceived quality of life (QoL). Up to 50% of people with MND can present with cognitive and behavioural impairment, with an associated increase in caregiver burden or strain. However, there has been no systematic exploration of the relationship between QoL and cognitive or behavioural impairment in MND.

Aim: The aim was to explore if there is a relationship between QoL and cognitive/behavioural impairment in MND, while also supplementarily looking to determine the types of cognitive/behavioural and QoL measures utilised in these studies. Methods: A PROSPERO registered systematic search was performed across multiple databases (PsychINFO, Embase, Medline, AMED) on February 22, 2023. Studies utilising quantitative methods of measuring QoL, cognitive/behavioural functioning/impairment were included. Findings examining relationships between QoL-cognitive/behavioural impairment were extracted and synthesised.

Results: A total of 488 studies were identified, with 14 studies included in the systematic review. All 14 studies were observational (11 cross-sectional, 3 longitudinal). Around 50% of studies utilised disease specific cognitive-behavioural measures, with almost all studies using variable, disease non-specific QoL measures. Study quality was variable, indicative of further robust research needed. Of 8 studies measuring behavioural impairment 62.5% (N = 5) found either a lower QoL difference or association. Only 33.3% (N = 4) of 12 studies measuring cognitive impairment found a lower QoL difference or association.

Conclusions: Behavioural impairment may have more impact on QoL in MND than cognitive impairment. However firm conclusions cannot be drawn as there is variability in the assessment of QoL and cognitive/behavioural impairment some of which was not appropriate for these patients. Future research should utilise disease specific, multidomain measures both cross sectionally and longitudinally to improve the quality of the research and elucidate the QoL-cognitive/behavioural impairment relationship.

323. The revised El Escorial Criteria (EEC) use in different countries

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Introduction – The revised El Escorial Criteria (EEC; (1)) are still used as part of the inclusion criteria to evaluate eligibility of ALS patients for enrolment into clinical trials. It is also known that "not meeting a specific El Escorial category is the most important reason for trial exclusion "(2). Therefore, a "correct" and consistent application of the EEC throughout study sites and countries is mandatory.

Methods - We analysed prospectively entered data from ALS Progeny database from Belgium (n=411), Ireland (n=79), Italy (n=252), the Netherlands (n=623) and Switzerland (n=298) that had at least two ALSFRS-R or vital capacity measurements and an El Escorial classification within 90 days from date of diagnosis. These data were analysed for the distribution of the different EEC categories in each country.

Results – The distribution of the EE Categories per country were significantly different ($p<0.001$) but diagnostic delay was not ($p = 0.14$). The category "probable laboratory supported" is not used in Ireland. While the category "definite" is applied more frequently in Ireland and less frequently in Belgium compared to other countries, the "probable* category is equally applied in all countries. However, the proportion of "probable lab supported" category is significantly higher ($p<0.001$) in Switzerland and Belgium compared to other countries. A survey revealed that only in these countries EMG is performed by neurologists who also examine the patients clinically.

Conclusion – A similar diagnostic delay but different distribution of EEC between various European centres suggests that EEC are applied differently. This has been shown previously in a round robin study design (3). The higher proportion of "probable lab supported" category in the countries Belgium and Switzerland suggests that neurologists also familiar with EMG are more confident in applying this category. Harmonisation and training of EEC application seems mandatory for site participation in clinical trials. Thus, the recruitment of ALS patients probably varies in the different countries, supporting the application of an individualized risk-based criterion as suggested by Van Eijk et al. (2).

324. TIDALS : Protocol for a Randomized, Placebo-Controlled, Double-Blind Phase II Trial of Safety and Efficacy of the GSK-3 inhibitor Tideglusib in ALS

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Introduction:

TDP-43 is the major proteinopathy, described in 90 % of ALS cases. Tideglusib is a GSK-3 inhibitor which can decrease TDP-43 levels and promote nuclear re-localization in human ALS lymphoblasts, as well as in the transgenic mouse model. Thus, this drug may be a promising disease-modifying candidate for the treatment of ALS.

Methods:

TIDALS is a multicenter, double-blind, randomized, placebo-controlled phase II trial of Tideglusib in ALS patients (NCT05105958). Safety and tolerability are the primary endpoints. Efficacy is a secondary endpoint and will be assessed by the change in ALSFRS-R and slow vital capacity. Patients will receive a daily weight-adjusted dose of Tideglusib, or placebo over a treatment period of 12 weeks. Regular assessments of safety will be performed throughout the treatment and in the follow-up period. Additionally, we will collect blood samples to assess target engagement and evaluate potential biomarkers of disease progression. A total of 98 patients with probable or definite ALS according to the revised El Escorial criteria and within 18 months of disease onset shall be included in 5 centers in Switzerland.

Discussion:

This study's primary objective is to determine the safety and tolerability of Tideglusib, a GSK-3 inhibitor with the potential to act on TDP-43 pathology, in ALS patients. The most common side effect of this drug that has been described is asymptomatic liver enzyme elevation. The major exploratory endpoint will be efficacy, assed by changes in the revised ALS-FRS and vital capacity.

If no severe side effects are observed and if this study shows a potential clinical benefit, this will justify a Phase III trial in a larger population.

325. Time from ALS symptom onset to key disease milestones for slow, intermediate, and fast progressors: a real-world cross-sectional survey

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Background: ALS is a heterogeneous neurodegenerative disease with mean survival of 3–5 years from symptom onset.

Aim: To estimate time from symptom onset to key disease milestones in people living with ALS (pALS) with slow, intermediate, or fast disease progression. These disease milestones reflect escalating healthcare resource utilization associated with disease progression and functional loss in pALS.

Methods: In the Adelphi ALS Disease Specific Programme™, a multinational cross-sectional survey (July 2020 to March 2021), neurologists completed questionnaires for pALS under their care including ALSFRS-R score and symptom duration; from this we calculated disease progression rate (48 minus ALSFRS-R score divided by symptom duration in months).

Results: The analysis included 867 pALS (mean age 61.3 years; 62.5% males). Disease progression was divided into tertiles (using cutpoints from this dataset) of slow (≤0.36 points/month), intermediate (0.36–0.77), and fast (≥0.77) rates. For slow, intermediate, and fast progressors, respectively, the mean (95% CI) time from symptom onset to first consultation was 5.4 (4.4–6.3), 3.6 (3.1–4.1), 2.7 (2.3–3.0) months and to diagnosis was 11.6 (9.4–13.8), 7.5 (6.8–8.2), 5.2 (4.7–5.7) months. The interval from symptom onset to employment change was 29.8 (22.0–37.6), 13.2 (11.4–15.0), 8.4 (7.1–9.7), need for walking aid 30.4 (25.4–35.4), 16.8 (15.1–18.6), 8.8 (8.0–9.6), caregiver 33.0 (26.0–40.1), 18.8 (17.2–20.4), 10.8 (9.9–11.8) and wheelchair 58.6 (27.6–89.6), 30.8 (27.7–33.8), 14.7 (13.3–16.1) months. Time to need for communication aid was 67.2 (22.8–111.7), 32.9 (28.2–37.6), 15.5 (13.8–17.3), respiratory aid 80.0 (48.8–111.2), 33.7 (29.9–37.4), 17.6 (16.0–19.3), gastrostomy 102.0 (44.5–159.5), 39.6 (34.4–44.8), 19.0 (17.2–20.8), eye gaze technology 74.4 (5.2–143.6), 35.4 (28.0–42.8), 13.2 (10.2–16.3) and care facility 75.4 (15.9–166.6), 36.3 (29.2–43.5), 18.2 (14.3–22.1) months.

Conclusions: In intermediate and fast progressing ALS, times to disease milestones were shorter than for slow progressing ALS, illustrating the heterogeneity of disease progression. Across the board, milestones were consistently reached earliest by those with the fastest disease progression rates and latest by those with the slowest rates. Effective therapies that slow disease progression may have the potential to reduce the burden of ALS and help pALS, physicians, and caregivers to better plan optimal care in relation to these ke

326. Tofersen in SOD1 ALS: the experience of Italian NeMO Centers.

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Twenty-seven ALS patients with SOD1 mutations were treated with intrathecal injections of Tofersen in the Expanded Access Program. Patients had different SOD1 variants, including c.73-4A>G, c.358-10 T>G, D12Y, H49N, N66S, P67L, H81R, L85F, G94D, I114F, D126G, E134del, L145F, G148C, I150T and D91A (homozygous and heterozygous state). The mean age of disease onset was 51 years (34-70 y). All patients had spinal onset, except one with bulbar signs as first symptoms. The mean interval of time between symptoms onset and starting therapy was 83 months (range 7-276 m). All patients were periodically evaluated by neurologist at four NeMO clinical centers in Italy. Four patients died during treatment period. One patient decided to discontinue due to perception of inefficacy. Fourteen of the remaining 22 patients were treated for at least one year and for this group clinical data regarding the 12 months prior to therapy were available. In seven patients (50%), the slope of MRC sum score and ALS-FRS-R showed a clear worsening over the 12 months prior the treatment, while over the one year of Tofersen administration less evident or no changes were observed. In the remaining 7 patients Tofersen did not appear to modify clinical course. Post-dural puncture headache was rarely reported. Notably, two patients showed a rapid deterioration of lower limbs muscular strength a few days after the 11th and 13th injection, respectively, without any sensory symptoms. CSF examination showed marked increase of proteins (> 200 mg/dl) and cells (>90 cells/mm3). Based on magnetic resonance imaging and EMG examinations, a pure motor radiculitis related to drug administration was diagnosed. Both patients improved markedly after steroid treatments. The identification of this side effect is fundamental, as it might be confused with the natural progression of the disease. Analysis of neurofilament light chain (NFL) in a subgroup of patients showed a reduction in serum concentration in most Tofersen-treated cases.

327. Tofersen Safety Experience From An Ongoing Global Expanded Access Program.

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(pref. Poster)

Tofersen is an intrathecally administered antisense oligonucleotide designed to reduce the synthesis of human superoxide dismutase-1 (SOD1) protein by inducing RNase H–mediated degradation of SOD1 messenger RNA. It is being developed as a potential treatment of ALS associated with a mutation in the SOD1 gene. Safety data from the randomized, placebo-controlled Phase 3 study and ongoing open label extension study of tofersen have been previously described.1 Neurologic serious adverse events (SAEs) of myelitis, radiculitis, aseptic meningitis, intracranial hypertension, and papilledema have been reported in participants who received tofersen 100mg. Most of these events were not treatment limiting and managed through standard of care. In July 2021, an expanded access program (EAP) opened to provide tofersen 100mg to people with SOD1-ALS not enrolled in a clinical trial, prior to commercial availability. Safety data are collected in the EAP.

As of 12 Jan 2023, 236 people with SOD1-ALS from 18 countries had enrolled in the EAP and had received up to 21 treatment cycles. 95 non-serious AEs and 53 SAEs were reported. The most common AEs were post lumbar puncture syndrome, back pain, headache, and procedural pain. The most common SAEs by MedDRA preferred term were pneumonia aspiration and respiratory failure. These AEs and SAEs are similar to those reported in the clinical trials [1].

Six participants experienced seven neurologic adverse events assessed as related to tofersen by the health care provider. These included two serious events of myelitis, two serious events of aseptic meningitis, one serious event of papilledema, one serious event of radiculopathy, and one nonserious event of lumbar radiculopathy. Papilledema and aseptic meningitis occurred in the same participant. No new safety concerns have been identified. The types of neurologic events reported in the EAP, their management, and their sequelae are consistent with the safety profile observed in tofersen clinical trials.

[1] Miller TM, et al. VALOR and OLE Working Group. Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N Engl J Med. 2022 Sep 22;387(12):1099-1110

328. Traditional respiratory measurement and the current state of the field

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Background

Pulmonary complications resulting from weakness of the respiratory musculature, are the leading cause of death in ALS [1]. The current gold standard for assessing respiratory function is spirometry, which provides a volume of air in and exhaled, with volume theoretically decreasing with weakness. However, this is physically demanding, requires a mouth/face seal and measurement is completed episodically rather than continuously.

Objective

To assess the commercial and research-based alternatives to the current clinical measures.

Methods

A scoping review was carried out on the clinical respiratory field. Google Scholar, PubMed, Scopus and general search engines were used to review the available literature. The following main keywords were used to identify papers of interest which were then assessed by the authors: (1) ALS, amyotrophic lateral sclerosis, MND, motor neurone disease; (2) respiratory measurement, rate, volume; (3) medical device; (4) neurology, neuromuscular diseases.

Results

Spirometry is the gold standard for respiratory function measurement, focusing on volume and rate of airflow. The main clinical alternative is body plethysmography. Novel technology based respiratory measurement devices are focused on two main categories: respiratory rate measurement, which is not useful in ALS and volume measurement. Remote respiratory monitoring is possible with digital spirometers while continuous monitoring has involved variations of respiratory inductance plethysmography technique (bands that fit around the rib cage and abdomen respectively). There is no commercial wearable respiratory volume device available.

Discussion

There is an unmet need in respiratory health for a wearable, remote device that can record respiratory volume measurement continuously. The only other volume measurement device are bands that fit around the chest. These are bulky, prone to slippage and are rarely used out the research setting. Spirometry is only able to record function at one point in time and remote spirometry still requires remote assistance. A small wearable sensor will improve patient care and monitoring for high risk populations.

References

[1] L. Aboussouan, “Respiratory disorders in neurologic diseases,” Cleveland Clinic journal of medicine, vol. 72, pp. 511-20, 07/01 2005, doi: 10.3949/ccjm.72.6.511.

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329. Treatment with acetyl-l- carnitine: new prospective from an observational retrospective study

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ALCAR (gamma-trimethil-beta-aminobutyrobetaine) is the acetyl ester of carnitine, it is a donor of acetyl groups and increases the intracellular levels of carnitine, the primary transporter of fatty acids across the mitochondrial membranes. In vivo studies showed that ALCAR ameliorates mitochondrial dysfunction, restores synaptic transmission and exerts protective effects against neuroinflammation. Consequently, decreased oxidative stress markers and pro-inflammatory cytokines have been detected. In a previous double-blind placebo-controlled phase II trial 80.9% of participants receiving ALCAR and 97.5% receiving placebo became non-self-sufficient (p=0.0296). Mean ALSFRS-R scores at 48 weeks were 33.6 (SD 10.4) and 27.6 (9.9) (p=0.0388), and mean FVC scores were 90.3 (32.6) and 58.6 (31.2) (p=0.0158) respectively in active and placebo arms.

We conducted an observational, retrospective, multicentre, case-control study to provide additional data on the use of ALCAR in ALS in clinical practice.

Not treated subjects matched (by age at diagnosis (+/- 5 years), site of onset, disease duration (+/- 3 months) at baseline) with subjects treated with ALCAR 1.5g/day or 3 g/day were included (45 subjects per group).

ALCAR 3g/day vs placebo: Among not treated subjects 22 (48.9%) were still alive at 24 months after baseline, as compared to 23 (51.1%) among treated subjects (p=0.8330, adj. OR 1.18, 95% CI 0.46-3.02); at 12 months after baseline 7 not treated subjects (16.7%) were self-sufficient, as compared to 2 (4.7%) treated subjects (p=0.0719). No statistically significant differences were detected in ALSFRS nor FVC evaluation among subjects treated with ALCAR 3g/day vs those not treated.

ALCAR 1.5g/day vs placebo: Among not treated subjects 22 (48.9%) were still alive at 24 months after baseline, as compared to 32 (71.1%) among treated subjects (p=0.0314, adj. OR 0.27, 95% CI 0.10-0.71). At 12 months after baseline 4 not treated subjects (9.5%) were self-sufficient, as compared to 3 (7.7%) treated subjects (p=0.9999). For ALSFRS-R, a mean slope of -1.0 was observed in treated subjects compared to -1.4 in those not treated (p=0.0575). No statistically significant difference was detected in the FVC evaluation.

Additional data should be provided to confirm the efficacy of the drug, demonstrate the effects of treatment on selected pharmacodynamic biomarkers implicated in the pathophysiology of ALS, and provide a dosage rationale.

330. Two novel SOD1 gene variants with aggressive ALS phenotype

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Introduction

Amyotrophic lateral sclerosis (ALS) incidence and phenotypic heterogeneity is due to an interaction between genetic background and differently distributed environmental factors. We investigated the effect of living near specific croplands on ALS risk, site of onset, age at onset and progression rate.

Methods

In a population-based dataset of ALS patients (PARALS registry), diagnosed between 2007 and 2014, we recover the historical residence in the 20 years before disease onset. We gather data on the geographical distribution of croplands in the same period. For all the municipalities we calculated the percentages of area covered by each crop, comparing them to patients smoothed incidence using linear regression. Then, we calculated proximity scores by assessing the percentage of area covered by each crop enclosed in a circle centered on the residence address (radii range 100-2000 meters, Figure 1), using historical residence data, weighting each exposure by the residence period.

Results

ALS cases incidence increased according to the percentage of area covered in each municipality by arable crops, ranging from 0.75 to 1.81 cases/100.000/year ($R=0.191$, $p<0.001$, Figure 2). Using historical residential data, arable crops and vineyards proximity significantly influenced age at onset, even considering different radii (for arable lands 100-1500 meters, for vineyards 500-2000 meters) and stratifying for sex, site of onset and genetic status. We found no significant effect on progression rate or site of onset.

Conclusion

We confirmed a higher ALS risk in municipality with high percentage of arable crops. Arable crops and vineyards proximity significantly reduced median age at onset.

331. Untangling a diagnosis with overlapping phenotypes: Late Onset Tay-Sachs disease and two sporadic ALS cases in one family.

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Amyotrophic lateral sclerosis (ALS) is a rare disease in the family of neurodegenerative disorders characterized by progressive loss of muscle control. Due to the overlapping phenotypes and clinical manifestation, it is challenging to estimate a definitive diagnosis, and it may often get misdiagnosed with other neuromuscular, metabolic, or psychiatric diseases.

One way to help navigate diagnostic accuracy relatively quickly and efficiently is whole exome/genome sequencing.

Here we report a case of three family members who presented ALS-like phenotypes with progressive upper and lower motor neuron degeneration, affecting predominantly distal limb muscles, and the bulbar region as well. As genetic etiology seemed highly plausible, two of the patients were examined for a neuromuscular and neurodegenerative panel; surprisingly, the outcome of the gene panel was negative in both patients.

Therefore, patients were then enrolled in the study for Whole Exome Sequencing and underwent additional clinical observations. Interestingly, the proband was identified as a compound heterozygote for variants c.805G>A + c.1510C>T in the hexosaminidase A gene (HEXA), causing Late Onset Tay-Sachs disorder. Tay-Sachs is a rare inherited metabolic disease characterized by a deficiency in the ß-hexosaminidase A enzyme, leading to the toxic accumulation of gangliosides in neurons of the brain causing neurodegeneration. Based on additional clinical observations in the metabolic center, the enzymatic activity of ß-hexosaminidase A was tested, and showed decreased activity of the enzyme in the serum, plasma, and leukocytes. Sanger sequencing confirmed findings from whole exome sequencing and was extended to other family relatives. However, the two other family members with almost identical ALS-like phenotype (one being a maternal relative and the other a paternal relative of the proband) did not carry any of the detected mutations, and after detailed whole exome analysis, they remained classified as two mutually unrelated patients with sporadic ALS.

This further proves the advantage of a detailed and thorough investigation of the genome beyond the known neuromuscular or neurodegenerative gene panel for accurate diagnosis and potential targeted therapy.

332. Validity and reliability measures of the Swedish version of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS)

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Background: Cognitive and behavioral impairment is observed in up to 50% of patients with amyotrophic lateral sclerosis (ALS). The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) is an ALS-specific, multi-domain screening tool. Although the ECAS is available in Swedish (S-ECAS), it has not yet been validated in Sweden.

Objectives: Assess validity and reliability of the S-ECAS Version A.

Methods: The study included 176 patients with ALS or other motor neuron disease diagnosed between September 2017 and October 2021 at the Karolinska ALS Center in Stockholm, Sweden, and 35 age-matched healthy controls. S-ECAS was validated against the Montreal Cognitive Assessment (MoCA) and optimal cut-offs, receiver operating characteristic (ROC) cure and area under the curve (AUC) were calculated.

Results: We identified an optimal cut-off of 108 for the S-ECAS total score and 82 for the S-ECAS ALS-specific score to detect cognitive impairment S-ECAS showed to be performant in indicating abnormal cognition with an AUC of 0.73 for S-ECAS ALS-specific score and 0.77 for S-ECAS total score. There was good internal consistency with a Cronbach's alpha of 0.79.

Conclusions: The study demonstrates good validity and performance indices for S-ECAS- version A for the detection of cognitive dysfunction in Swedish newly diagnosed ALS patients.

333. Volumetric Analysis of the Brainstem: Predictability of Respiratory and Bulbar Function in Amyotrophic Lateral Sclerosis

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons in the brain, brainstem and spinal cord, eventually leading to respiratory failure. As for the management of respiratory complications in ALS patients, non-invasive ventilation (NIV) has been shown to improve survival and quality of life. However, the lack of a reliable prognostic model to predict the need for non-invasive ventilation has hindered the development of clinical practises and trials.

Objectives

The primary objective of this study is to assess whether brainstem volumes can predict the need for non-invasive ventilation. A secondary objective is to investigate the correlation between brainstem structure volumes and respiratory and bulbar functions assessed with standardized tools.

Methods

This study included 41 ALS patients from the Paris cohort of the PULSE study (protocol 2013-A00969-36). Volumetric analysis of brainstem regions using T1-weighted images was performed. Clinical and spirometric data were collected at three time points: at baseline, then after three and six months.

Results

A nominal logistic regression model that included brainstem volumes predicted the need for non-invasive ventilation over a six-month period with a sensitivity and specificity of 85%. Brainstem volumes correlated significantly with the ALSFRS-R bulbar sub-score and expiratory and inspiratory functions assessed by spirometry respiratory volumes and cough peak flow.

Discussion

The analyses also confirmed the predictive model's potential, incorporating brainstem volumes at baseline, to discriminate between patients requiring non-invasive ventilation at six months. Combining these parameters with spinal cord imaging may provide a reliable prognostic biomarker for bulbar and respiratory function deterioration in ALS.

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334. Worldwide geographical distribution of clinical trials in amyotrophic lateral sclerosis based on information available in clinical trial databases

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Introduction: Clinical trials location is determined by many factors, including the availability of patient populations, regulatory environment, scientific expertise, and cost considerations. In the study of amyotrophic lateral sclerosis (ALS), where genetic differences have been described and may be related to geographic setting, this could have implications for the clinical interpretation of results in underrepresented geographic settings.

Aims: Describe the situation on potential disparities between countries in ALS clinical research based on data available from clinical trial registries.

Methods: We reviewed available information about clinical trials on ALS in ClinicalTrials.gov (CT), EU clinical trials register (EUCTR) and WHO International Clinical Trials Registry Platform (ICTRP). Search criteria were clinical trials in ALS, status recruitment and active not recruiting in phase 2 and 3. Studies were classified according to type of study, active treatment comparator and participating countries.

Results: The total number of clinical trials identified were 67 in ClinicalTrials.gov, 59 in EUCTR (57 drugs, 2 medical devices) and 63 in ICTRP (59 drugs, 4 medical devices). The type of study that was most performed was parallel assignment (45/67), followed by single group assignment (19/67) and sequential assignment (3/67). Active comparators in parallel assignment were placebo (40/45), riluzole (2/45) and placebo plus riluzole (3/45).The drugs with most studies were edaravone, MT1186 (4) y FAB122 (1). The most countries with most studies conducted were the US with 38 studies (56%), Canada 16 (23%), Germany 16 (23%), the UK 13 (19.4%), the Republic of Korea 7 (10%). Spain had 10 studies that were active (14%). Accordingly, geographical regions distribution were: East Asia 32%, Europe 54%, Middle East 9%, North America 66%, North Asia 9%, Pacific 21%, South America 6%. We found diversity in the age ranges for admission to clinical trials: highlighting 18 years and older (38/67) and 18 years to 75 yo (9/67) .

Conclusion. The data obtained in our review show, despite some discrepancies in the registries consulted, a non-homogeneous distribution in clinical trials at international level, this may influence on the interpretation of the results obtained. Encouraging the participation and higher number and diversity of countries in clinical research is likely to increase the study of the different subgroups of patients associated with geographic variability.

335. Yentl's syndrome, a real phenomenon in Amyotrophic Lateral Sclerosis (ALS)?

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INTRODUCTION:

ALS is a neurodegenerative disease with a variable incidence and prevalence according to the different databases consulted, being PRO-ACT the largest publicly available repository of combined ALS clinical trial data. There is an estimated male-female ratio in favour of men at younger ages, which tends to equalize with ageing. It is possible that this higher male prevalence results in a higher inclusion of men in clinical trials which could lead to biases in the observed results, as it would not allow the analysis of differences between sexes. Our aim is to describe the demographics dates of the population included in ALS clinical trials in the last 8 years at national reference centers, with special interest in female participation.

METHODOLOGY:

Retrospective and descriptive observational study using databases of national reference centers.

RESULTS:

After analyzing the databases of 4 hospital centers with a total number of 642 subjects, a greater participation of the male sex was evident in all the studies evaluated, representing 63.70% of the subjects included. This predominance has not varied significantly over the last 8 years. Our results correlate with the data published in PRO-ACT to date, where men represent 60% of the total number of participants.

CONCLUSION:

The predominance of the male sex in ALS clinical trials is a consistent and invariable finding, already demonstrated in other pathologies, and is known as Yentl's syndrome. This phenomenon prevents the principle of neutrality of medicine, allowing for a purely partial knowledge.

336. Prevalence of Oropharyngeal Dysphagia and its perception in ALS patients.

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Introduction: Oropharyngeal Dysphagia (OD) is a common disorder among ALS patients. An early diagnosis can present of respiratory and nutritional complications. Even though it is highly recognized in bulbar phenotype of ALS, it is also present in some spinal phenotype. The aim of this study is to diagnose oropharyngeal dysphagia and to compare with the perception of the symptoms by the patients with ALS by phenotype.

Methods: We included 19 participants (60 ±10;, 13 women). 4 bulbar-onset and 15 spinal- onset (4 of them with bulbar affection at the beginning).
All participants were asked to complete the SWAL-QoL and were diagnosed with videofluoroscopy (VFS) at the time of the diagnosis and 4 months later. SWAL-QoL was considered as positive perception of OD signs if scored ≤ 3, and all VFS were analysed for finidings of safety and/efficacy impairment during swallow.

Results: Bulbar-onset participants were found a positive diagnose for OD and all expressed having symptoms. 37% of Spinal-onset participants were diagnosed with OD in both or the second exploration and 57% of them had bulbar signs. Just 2 spinal-onset with no bulbar signs explained an impaired swallow perception whereas the VFS was normal.

Conclusion: OD is more prevalent in bulbar-onset ALS patients or when bulbar signs appear. Most of patients with OD state signs of difficulty or discomfort with swallow. Hence, SWAL-QoL is a good questionnaire to assess the perception of swallow complications in ALS patients.

Rapid fire presentations

337. Deletion of endothelial TDP-43 disrupts the vascular barrier triggering inflammation and hemorrhages in the central nervous system

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Defects in vascular growth and stability are common features in many pathological processes, including neurodegenerative diseases. The molecular alterations contributing to vascular defects in neurodegenerative disorders are not fully understood. TDP-43 is a DNA/RNA-binding protein that regulates gene expression and its malfunction in neurons has been causally associated with multiple neurodegenerative diseases. Although progress has been made in understanding the functions of TDP-43 in neurons, little is known about its role in endothelial cells (ECs), angiogenesis and vascular homeostasis.

We generated endothelial-specific and inducible TDP-43 knockout mice and studied the role of TDP-43 in retinal angiogenesis and vascular homeostasis using immunostaining techniques. The molecular mechanisms underlying the in vivo phenotypes were elucidated by knocking down TDP-43 in cultured human ECs.

In maturing vessels of the central nervous system, loss of TDP-43 results in altered actin cytoskeleton organization, disorganized distribution of cell-cell junction proteins and impaired vascular barrier integrity. and, consequently, hemorrhages and inflammation in the retina, brain and spinal cord. Cultured TDP-43-depleted ECs show reduced stable adherens junctions and altered cell-matrix adhesion sites. Mechanistically, loss of TDP-43 leads to increased actomyosin contraction, preventing proper formation of cell-cell and cell-matrix adhesions.

Our results indicate that TDP-43 is essential for the formation of a stable and mature vasculature and identify endothelial TDP-43 as an important regulator of vascular barrier function, contributing to cell-cell junction integrity.

338. Haploinsufficiency of C9ORF72 selectively impairs autophagy in C9ORF72-linked ALS.

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One of the most common genetic mutations associated with familial Amyotrophic lateral sclerosis (ALS) and Fronto-temporal dementia (FTD) has been identified as the hexanucleotide repeat expansion of GGGGCC (G4C2) in the intron of the gene chromosome 9 open reading frame 72 (C9ORF72). Although the underlying mechanisms of ALS onset are unknown, new insights implicate altered proteome homeostasis as a fundamental process underlying ALS pathogenesis. Motor neurons are intrinsically vulnerable to proteome stress. Unfolded or misfolded proteins are normally cleared by the various cell's clearance machinery such as the Ubiquitin proteasome system (UPS), ER-associated degradation and autophagy. C9ORF72 protein interacts with SMCR8 (Smith–Magenis syndrome chromosomal region candidate gene 8) and WDR41 (WD40 repeat-containing protein 41) to form stable CSW complex, which acts as a GDP/GTP exchange factor for Rab proteins, resulting in autophagy regulation. TANK Binding Kinase, TBK1, a serine-threonine kinase, phosphorylates SMCR8. Loss of function mutations in TBK1 were found to be associated with familial and sporadic ALS. Similarly, haploinsufficiency of C9ORF72 has been well established as one of the pathogenic mechanisms in C9ORF72-linked ALS. These findings led us to examine the interplay between C9ORF72 expression, and regulation of autophagy in rodent and human models of C9ORF72-ALS.

339. Elucidating the timing of TDP-43 related phenotypes using iPSC-derived motor neurons from TARDBP ALS patients

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal adult-onset neurodegenerative disease, characterized by the selective loss of both upper and lower motor neurons. In 3-7% of familial patients, the disease is caused by inherited mutations in the Transactive response DNA binding protein (TARDBP) gene, encoding the TDP-43 protein. However, cytoplasmic hyperphosphorylated and ubiquitinylated TDP-43 protein aggregates are found in neurons and glial cells of 97% of ALS patients, including in patients with a hexanucleotide repeat expansion in Chromosome 9 open reading frame 72 (C9orf72) and all sporadic cases. Therefore, TDP-43 pathology is considered as a pathological hallmark of ALS. Our laboratory has previously shown that iPSC-derived spinal motor neurons (sMNs) harboring a heterozygous mutation in the TARDBP gene also display TDP-43 pathology with cytoplasmic mislocalization, C-terminal cleavage and accumulation of insoluble TDP-43. Additionally, axonal transport deficits have been observed. In this study, we now aim to elucidate the timing and order in which each of these phenotypes occur. To this end, live cell imaging, immunocytochemistry and western blot analysis are performed on TARDBP mutant sMNs, as well as their isogenic controls, at multiple timepoints during sMN differentiation. Preliminary results indicate that mitochondrial axonal transport is already impaired at the first timepoint, whereas changes in TDP-43 solubility and C-terminal cleavage are only observed at a later stage. These findings suggest that deficits in axonal transport might precede the accumulation of insoluble TDP-43 and C-terminal cleavage fragments.

340. Dynamic Translational Profile of Stressed iPSC-MNs from C9orf72-ALS Patients by Translating Ribosome Affinity Purification (TRAP)

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Background: The G4C2 Hexanucleotide Repeat Expansion (HRE) in the gene C9orf72 is the commonest genetic cause of ALS. The intronic repeat RNA accumulates intracellularly as RNA foci and is translated non-canonically into dipeptide repeat proteins (DPRs), both of which have been shown to affect RNA metabolism. Furthermore, impaired global translation and perturbed stress granule dynamics have been associated with C9orf72-ALS. We hypothesize that the C9orf72 HRE may lead to a difference in the profile of translating mRNAs (translatome) in motor neurons at baseline, after stress or during recovery.

Methods: In this study, we treated human induced Pluripotent Stem Cell-derived Motor Neurons (iPSC-MNs) from 3 healthy controls (HCs) and 3 C9orf72-ALS patients with 0.5 mM sodium arsenite (ARS) for one hour, and obtained the transcriptome and translatome at baseline, immediately after stress, and after 2 h of recovery. To validate the findings from RNA sequencing, we characterized the ARS-induced stress response for up to 24 h after stress removal, through a range of biochemical experiments.

Results: We found a similar transcriptomic profile in C9orf72-ALS iPSC-MNs compared to HCs after stress. However, a discrete group of 68 Differentially Expressed Genes (DEGs) were identified in the translatome of C9orf72-ALS iPSC-MNs after 2 h of recovery. Notable DEGs relevant to ALS pathogenesis include UNC13A and PURA, and GO term analysis of the DEGs shows enrichment in synaptic function and neuronal projection. Gene Set Enrichment Analysis (GSEA) suggests an upregulation of antioxidant activity, apoptosis, translation and ribosome biogenesis, and a downregulation of synapse organization, axonal development and cellular signalling in C9orf72-ALS iPSC-MNs during early recovery. However, we did not detect an abnormal stress response in the disease lines in terms of cell viability, immunoblotting of apoptotic, autophagic and global translation activity markers, and stress granule dynamics. We also examined C9orf72 protein expression and the nuclear/cytoplasmic ratio of TDP-43 at different time points after stress and did not see a significant difference between C9orf72-ALS and HC.

Conclusions and plans: The translatome of C9orf72-ALS iPSC-MNs reveals a number of dysregulated genes and pathways during early recovery from transient ARS treatment. Future experiments will investigate the spatial association of selected DEG mRNAs with C9orf72 HRE foci and stress granule prote

341. MAM lipidome changes associated with TDP-43 dysfunction

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INTRODUCTION: TDP-43 may contribute to ALS pathogenesis in different cellular compartments like mitochondria. Mitochondria show intimate contact with the particular endoplasmic reticulum (ER) membrane subdomains termed mitochondrial-associated membranes (MAMs). MAM lipid composition is essential for its proper function. Consequently, changes in their lipidome could compromise the activity of proteins residing in MAMs. Our previous finding that TDP-43 alterations in animal and cellular models are related to changes in MAM activity led us to assess the possible relationship between TDP-43 (dys)function and MAM lipidome.

OBJECTIVE: To evaluate the potential changes in MAM lipidome secondary to TDP-43 alterations and establish a relationship between TDP-43 and lipid metabolism.

METHOD: We evaluated the lipidome of MAM and ER in the human frontal cortex (n=5 control group; n=6 ALS group) and tissue from transgenic B6N-Cg-Tg(Prnp-TARDBP*Q331K)103Dwc/J (TDP-43 Q331K) mice, both in brain and spinal cord samples. After subcellular fractionation, we used liquid chromatography-mass spectrometry (LC-MS) to perform an untargeted lipidomic approach. To assess the potential alterations in MAM-controlled lipid metabolism related to TDP-43 dysfunction, we have also evaluated phospholipid metabolism in a human cellular model of TDP-43 loss of function.

RESULTS: Untargeted lipidomics analysis shows that MAM and ER lipid composition is different in humans and animals. In addition, we also observed that alterations in TDP-43 affect part of MAM and ER lipidome in all of the analyzed samples. In contrast, the decrease of TDP-43 levels in HeLa-PLKO cells does not lead to global changes in phospholipid metabolism, suggesting that extra-MAM lipid metabolism (Kennedy pathway), could compensate for potential loss of TDP-43 controlled pathways.

CONCLUSION: Abnormalities in TDP-43 can affect the lipid composition of MAM and ER, both in human and animal models of mutated TDP-43. Changes in MAM composition may affect proteins that reside in them, which would partly explain previous results in which we have observed alterations in MAM activity in models of TDP-43 dysfunction.

342. Gap junctions are functionally enhanced in iAstrocytes derived from C9ORF72 repeat expansion patients

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INTRODUCTION: ALS is an incurable neurodegenerative disease, in which the C9ORF72 repeat expansion (C9RE) is a major causal genetic impairment. The same mutation gives rise to FTD, placing the C9RE at the centre of an ALS-FTD spectrum. Astrocytes are important non-neuronal cell types that provide functional and structural support to neurons but become toxic in ALS-FTD. In vitro, astrocytes exhibit toxicity through intercellular communication and secreted factors, thus indicating a source of dysfunction at the astrocyte membrane. The mechanisms of astrocyte toxicity and dysfunction remain to be fully understood. Here we have electrophysiologically investigated membrane dysfunction in C9RE astrocytes.

METHODS: Enriched in vitro cultures of S100β+ astrocytes were generated from fibroblasts using a direct induction protocol (iAstrocytes). Fibroblasts were obtained from healthy subjects and patients living with C9RE. Whole-cell patch-clamp electrophysiology was used to characterise iAstrocyte membrane function.

RESULTS: Intrinsic astrocyte membrane currents were evoked using a voltage-step protocol and current-voltage relationships established for each iAstrocyte line. For C9RE iAstrocytes, the data showed a significant gain-of-function enhancement in passive current amplitudes versus control iAstrocytes (3-fold increase in C9RE compared to controls; $p<0.0001$, Two-way ANOVA with Tukey's tests). Importantly, the differences in intrinsic membrane properties indicate that the observations are not due to accelerated maturation (rectification index of passive currents shows no consistent differences between C9 and controls, $p\geq0.08$), but an increase in the expression of a specific ion channel/transporter in C9RE iAstrocytes. To explore this we pharmacologically characterised the evoked membrane currents of each line. Using selective pharmacology we have determined that the gain-of-function in C9RE iAstrocytes is directly associated with the up-regulation of gap junctions (10µM CBX significantly decrease current amplitude, $p<0.0001$, Two-way ANOVA with Tukey's tests).

CONCLUSIONS: C9RE iAstrocytes display gain-of-function membrane current enhancement due to an upregulation of gap junction membrane channels. Gap junction expression is directly associated with the transport of molecules that impact on neuronal viability. Our work will now investigate the impact of C9RE iAstrocytes on the viability of neurons and the mechanisms giving rise to these alterations.

343. Targeting De Novo Fatty Acid Synthesis as a Therapeutic Strategy to Alleviate Non-cell Autonomous Mechanisms in ALS

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive degenerative motor neuron disease with no effective treatment. Exploration of lipid metabolism has led to the identification of descriptive biomarkers of disease, providing insights into the underlying biological processes involved in a diverse range of psychiatric and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Clinical and experimental studies have also highlighted the significance of lipids in the neurodegenerative process of ALS. In fact, several lipid-lowering approaches have been tested in the disease, showing promising results. Our findings reveal that the progression of ALS is associated with a dysregulated elongation of long-chain fatty acids in serum samples from two distinct follow-up patient cohorts. Lipotoxicity induced by glial cells via elongation of saturated fatty acids has been recently identified as a potential mediator of neurodegeneration, and inhibiting this process has been proposed as a neuroprotective therapy. Our research indicates that TDP-43 or FUS loss-of-function in both muscle and glial cells, is implicated in the dysregulation of elongation of saturated fatty acids. In addition, inhibition of long-chain fatty acid elongation, achieved through both genetic and pharmacological means using a potent, selective, and orally bioavailable inhibitor, consistently leads to improved neuromuscular phenotypes and increased life expectancy in fruit flies with muscle-specific TDP-43 deficiency. To summarize, we propose that therapeutic interventions aimed at reducing the accumulation of saturated fatty acids by inhibiting their de novo synthesis, could alleviate the non-cell autonomous degenerative mechanisms that supportive cells exert on vulnerable motor neurons in ALS.

344. Epigenetic analysis on organoids for the study of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease (NDD) with a progressive clinical curse which affects upper and lower motor neurons (MNs), causing weakness, muscle atrophy and spasticity. As for other NDDs there are two typical forms of ALS, the sporadic (sALS), which account for 90% of the cases, and the familial one. Until now, only symptomatic treatments are available, especially for the lack of realistic models which can mimic the physiological environment in which cells grow. One important innovation are organoids. Organoids are pluripotent stem cell-derived self-organizing structures which can recapitulate the tissue of origin in vitro. Brain organoids contain both neural and glial cells and are used for disease modeling, i.e. for the study of cells interactions and of neurodevelopment.

In our laboratory, we already optimized a protocol for motor neuron organoids (MNOs) formation from CTRL and sALS NSCs. Aim of this project was the investigation of the epigenetic characteristics of organoids at each differentiation step, neural stem cells organoids (NSCO), motor neuron progenitors organoids (MNOPs) and MNOs and the comparison of these characteristics which the ones of 2D cultured cells. We performed an ELISA assay for DNA methylation status on 5-methylcytosine (5-mC) finding a decreased methylation ($p^{*}<0.05$) in sALS MNOs when compared to 2D MNs. Moreover, we performed western blot analysis to test the expression of two key methylating enzymes, the DNA methyltransferase 1 (Dnmt1) and the DNA methyltransferase 3a (Dnmt3a). We found a significant decrease in the protein expression of Dnmt1 in both CTRL and sALS MNPOs and MNOs when compared to the corresponding NSCOs, whereas we did not find any significant difference in Dnmt3a expression. Moreover, we are evaluating the gene expression of Dnmt1 and Dnmt3a by RT-qPCR and we found some differences both between organoids and 2D cultured cells and between CTRL and sALS cultures. Finally, we performed western blot analysis to test the methylation status of two sites involved in ALS pathology, lysine 9 and lysine 27, both on histone 3, finding an increasing trend of their protein expression during the differentiation protocol.

In conclusion, we confirmed the major deregulation of MNOs when compared to 2D cultured cells, already seen by our group through RNA-seq, and the possibility to use organoids as a useful tool for the study of epigenetics in ALS.

345. Cognate microglia – T cell interaction induce neurotoxic T cell function in a fast-progressing C9orf72 ALS animal model

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Protein aggregation and neuroinflammation are the two hallmarks of neurodegenerative diseases. ALS and FTD are progressive fatal neurodegenerative diseases with overlapping clinical symptoms and neuropathological findings. The hexanucleotide repeat expansion (G4C2)n in the C9orf72 locus is the most common genetic cause of ALS/FTD and has two main pathological consequences: a loss-of-function effect causing C9orf72 haploinsufficiency and toxic gain of function due to bidirectional transcription of (G4C2)n repeat. In the CNS of a fast-progressing C9orf72 animal model, congenic expression of poly-GA in neurons elevated microgliosis and pro-inflammatory interferon responses. We noticed a robust accumulation of CD3+ T cells during disease progression in the brain of C9orf72 transgenic mice. Cognate microglia – T cell interaction was suggested by histology and flow cytometry. Furthermore, whole-body depletion of CD3+ T cells resulted in higher body weight, prolonged life expectancy, and reduced behavior deficits, suggesting a neurotoxic function of accumulated CD3+ T cells in the brain of C9orf72 transgenic mice. Collectively, our data suggest a neurotoxic interaction of microglia with CD3+ T cells in C9orf72 ALS/FTD.

346. An interaction between synapsin and C9orf72 regulates excitatory synapses and is impaired in ALS/FTD

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Introduction

Synaptic dysfunction and degeneration of synapses are pathophysiological hallmarks of neurodegenerative diseases, including amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). The main genetic cause of ALS/FTD is a GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene (C9ALS/FTD) which leads to reduced expression of the C9orf72 protein. How C9orf72 protein haploinsufficiency impacts on neuronal function and contributes to C9ALS/FTD pathology is still unknown.

Here we reveal that C9orf72 plays a novel cell-autonomous role in the regulation of synaptic vesicle pools and neurotransmission at excitatory synapses. Our data propose that C9orf72 haploinsufficiency is a major contributor to synaptic dysfunction in C9ALS/FTD.

Methods

We investigated the role of C9orf72 at the synapse in vitro and in vivo using molecular biology, protein biochemistry, high resolution fluorescence and electron microscopy as well as electrophysiology techniques.

Results

We identified the synapsin protein family as new endogenous interactors of C9orf72 at synapses and mapped the interaction to the N-terminal longin domain of C9orf72 and the conserved C-domain of synapsin. Synapsins are the most abundant family of synaptic vesicle proteins which modulate neurotransmission by regulating synaptic vesicle pools.

Functionally, C9orf72 deficiency reduced the number of excitatory synapses and decreased synapsin levels at remaining synapses in vitro in hippocampal neuron cultures. Similarly, synapses were reduced in a gene-dosage-dependent manner in vivo in the hippocampal mossy fibre system of heterozygous and homozygous C9orf72 knockout mice. Consistent with synaptic dysfunction, electrophysiological recordings in hippocampal neuron cultures with reduced C9orf72 expression revealed impaired excitatory neurotransmission and network function, which correlated with a severe depletion of synaptic vesicles from excitatory synapses in the hippocampus of C9orf72 knockout mice. Finally, neuropathological analysis of post-mortem sections of C9ALS/FTD patient hippocampus with C9orf72 haploinsufficiency revealed a marked reduction in synapsin.

Conclusions

Thus, our results indicate that disruption of the interaction between C9orf72 and synapsin may contribute to ALS/FTD pathobiology.

347. Cortical network dysfunction in ALS using task-free magnetoencephalography

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ALS involves complex primary pathological and compensatory dysfunction of cerebral networks. Callosal (inter-hemispheric pathway) pathology has been a consistent observation in histological and MRI studies in ALS. Magnetoencephalography (MEG) is a brain imaging technique which non-invasively measures the micro-magnetic fields generated by oscillatory brain activity. Modern computational techniques allow analysis of this data to provide highly temporally and spatially localised information about cortical neurophysiology. Task-free, resting-state MEG offers unique insight into cerebral pathophysiology by revealing dynamic, network-level changes in oscillatory brain power and connectivity.

Thirty-six non-demented patients with apparently sporadic ALS and 51 age- and gender-matched controls underwent an 8-minute resting-state MEG recording and structural MRI scan. We calculated oscillatory cortical power, connectivity and complexity measures in 52 regions and 5 canonical frequency bands (δ, θ, α, β, γ). We used maximum statistic permutations tests to look for differences between groups, and the effect of disability (ALSFRS) on brain function, correcting for age, gender and handedness.

ALS patients showed decreased beta power in sensorimotor regions (p=0.034) and increased gamma power in frontotemporal regions (p=0.015). Frontotemporal activity complexity was also increased in ALS patients (p=0.008). Higher levels of ALS patient disability were associated with increased power in these same regions (p=0.007) and increased frontotemporal connectivity overall (p=0.005) but relatively more with the contralateral hemisphere (p=0.034).

A fall and regional shift of sensorimotor beta power is the consistent underpinning of ALS cerebral pathophysiology. Increased power, connectivity and complexity of brain activity in frontotemporal regions may reflect a distinct process within ALS and FTD's pathological spectrum, or compensatory response to primary motor system disintegration. Altered functional laterality may reflect specific damage to inter-hemispheric fibres of the corpus callosum. MEG may offer a biomarker for disease progression in ALS and secondary outcome measure in therapeutic trials. The extent to which MEG network changes occur pre-symptomatically requires dedicated exploration in carriers of pathological genetic variants.

348. isomiRs - a novel family of molecular biomarkers for ALS prognostication

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microRNAs are endogenous, non-protein coding, small RNAs. With Fratta and Malaspina labs we have reported the value of plasma microRNA-181 (miR-181) in ALS prognostication (Nature Neuroscience 2021). However, until recently, we were unable to analyze a much larger variety of microRNA isoforms, called isomiRs. Here, we develop a new bioinformatics technique for analysis of isomiRs. We reveal a novel panel of isomiRs, from a plasma cohort of 246 UK ALS patients, which are candidate ALS biomarkers with value in disease prognostication. Intriguingly, some of the isomiRs perform better as predictors than their canonical miRNA counterparts. Moreover, With Benatar, we were able to replicate our results for a specific isomiR of miR-339-5p in an independent American plasma cohort of 215 patients. This substantiate a first-of-its kind isomiR biomarker in ALS. This study features a conceptual and Innovative evaluation of a new family of molecular biomarkers in any disease. We hope that isomiRs will allow a more accurate ALS patient prognostication and promote clinical development.

349. Performance of serum neurofilament light chain in a wide spectrum of clinical courses of ALS – a cross-sectional multicenter study

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Objective: To assess the performance of serum neurofilament light chain (sNfL) in ALS in a wide range of disease courses – in terms of progression, duration, and tracheostomy invasive ventilation (TIV).

Methods: A prospective cross-sectional study at 12 ALS centers in Germany was performed. sNfL concentrations were age-adjusted using sNfL Z scores expressing the number of standard deviations from the mean of a control reference database and correlated to ALS duration and the ALS progression rate (ALS-PR), defined by the decline of ALS functional rating scale.

Results: In the total ALS cohort (n=1378) the sNfL Z score was elevated (3.04; 2.46-3.43; 99.88th Percentile). There was a strong correlation between the sNfL Z score with ALS-PR ($p<0.001$). In patients with long (5-10 years, n=167) or very

long ALS duration (≥10 years, n=94) the sNfL Z score was significantly lower compared to typical ALS duration of <5 years (n=1059) (p<0.001). Furthermore, in patients with TIV, decreasing sNfL Z scores were found in correlation with TIV duration and ALS-PR (p=0.002; p<0.001).

Conclusions: The finding of moderate sNfL elevation in patients with long ALS duration underlined the favorable prognosis of low sNfL. The strong correlation of the sNfL Z score with ALS-PR strengthened its value as a progression marker in clinical management and research. The lowering of sNfL in correlation with long TIV duration could reflect either a reduction in disease activity or in the neuroaxonal substrate of biomarker formation during the protracted course of ALS.

350. Ultrasound-mediated blood–spinal cord barrier opening prolongs survival in an ALS mouse model

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The limited efficacy of therapies in clinical development for Amyotrophic Lateral Sclerosis (ALS) may be linked to lack of drug penetration to the affected motor neurons due to the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB). This work was intended to circumvent this limitation by using a system to transiently open the BSCB. The safety and efficacy of repeated short transient opening of the BSCB by low intensity pulsed ultrasound (LIPU, sonication) were studied in an ALS mouse model. The BSCB was disrupted using a 1 MHz ultrasound transducer coupled to the spinal cord, with and without injection of insulin-like growth factor 1 (IGF1), a neurotrophic factor that has previously shown efficacy in ALS models. Results in healthy mouse models demonstrate that the BSCB can be safely disrupted and IGF1 concentrations significantly increased after a single session of transient BSCB disruption (176 ±32 µg/g vs. 0.16 ±0.008 µg/g, p<0.0001). Five repeated weekly sonications performed in ALS mice demonstrated a survival advantage in mice treated with IGF1 and ultrasound (US) compared to treatment with IGF1 alone (176 vs. 166 days, p=0.019), but also in mice treated with ultrasound alone vs untreated mice (178.5 vs. 166.5 days, p=0.018). Thus, these results suggest a survival benefit of ultrasound alone, independently of IGF1 administration. Histological analysis revealed a modulation of glial cell reactivity and an increased CD4+ T-cell infiltration in the spinal cord of mice treated by US+IGF1, compared to treatment with IGF1 alone. As CD4+ T-cells are known to be protective in this ALS mouse model, the mechanism underlying the survival benefit may be an immunomodulatory effect of US or US+IGF1. These results show the first step towards a possible beneficial impact of transient BSCB opening implicating immune cells for ALS therapy, as well as efficient BSCB opening for drug delivery.

351. Motor system connectivity in ALS: A corticomuscular magnetoencephalography study

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Introduction: Biomarkers of disease activity in ALS are needed to allow objective and sensitive detection of therapeutic benefit and accelerate the process of drug discovery. ALS is motor system disorder spanning cortex to muscle. Magnetoencephalography (MEG) is the most sensitive, non-invasive assessment measure of regional cortical neurophysiology. Corticomuscular coherence (CMC) reflects the functional coupling of cortical oscillations and downstream muscle activity in the search for a more holistic biomarker of motor system dysfunction in ALS.

Hypothesis: MEG-led measures of motor system functional connectivity can identify specific disrupted neural dynamics associated with ALS pathology.

Methods: In an ongoing study, data were available from 15 ALS patients and 15 healthy age-similar controls (HC). Participants underwent clinical evaluation with clinical assessments and a standardised MEG protocol involving a novel gripper task. Muscle contraction was measured using bipolar surface EMG recordings at both forearms. All participants performed 240 trials of the gripper task bilaterally, and 120 trials unilaterally on each side.

Results: During gripper-based muscular contraction, beta-band frequency CMC in the motor cortex was significantly reduced in ALS patients compared to healthy controls (cluster-based permutations between 8 – 25 Hz). There were no significant differences between absolute grip strength of the ALS patients and HC. Beta-band power differences were seen in the peri-response desynchronisation, and post-movement rebound. A reduction of CMC between cortex and contralateral muscle was also evident when considering its topographical distribution using localisation analysis. In both groups, coherence was localised to the same contralateral motor channels, but was considerably weaker in ALS disease compared to HC participants.

Conclusion: Beta-band CMC is a relatively easily acquired biomarker of early ALS motor system dysfunction. Albeit at the group level currently, there are immediate opportunities for its exploration as a secondary outcome measure in therapeutic trials, but in future work also as a potential pre-symptomatic biomarker in carriers of pathological variants linked to ALS.

352. Dysfunction Of Cortical Inhibitory Interneurons In Amyotrophic Lateral Sclerosis

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Background: Sporadic (s) and familial (f) forms of Amyotrophic Lateral Sclerosis (ALS) are characterized by perturbed excitation/inhibition (E/I) input balance to motoneurons. Previous studies using paired-pulse TMS (ppTMS) had shown that ALS is distinguished by a depressed Short-Intracortical Inhibition (sICI) and an enhanced Intracortical Facilitation (ICF). To shed light on mechanisms causing neuron excitability disorders, it is crucial to consider afferent interneurons that project onto UMN.1 2

Objective: Our aim is to investigate the degree of excitability of inhibitory interneurons projecting to UMN in early diagnosed sALS patients as compared to healthy controls (HS). Our research hypothesis is to find a perturbed sICI in ALS patients as compared to HS across different conditions (rest vs. tonic contraction).

Methods: Data were collected on 12 sALS patients and 16 HS. ppTMS was delivered over the primary motor cortex to evoke sICI in abductor digit minimi (ADM). To stress changes in inhibitory interneuron excitability, sICI was evaluated in different conditions: 1) Test TMS intensity was set at 1.2 of the resting motor threshold (RMT). Two intensities of conditioning TMS were tested (0.7 X RMT and 0.7 of the active MT, AMT) 2) sICI was compared at rest and during 10% of the maximal voluntary contraction.3 5

Results and Conclusions: The RMT was higher in sALS patients compared to HS. Given that test TMS intensity was stronger in sALS patients, the amplitude of the test motor evoked potential (MEP) was about 10% of the maximal motor action potential (Mmax) while it was about 5% Mmax in HS. Thus, the test MEP in ALS patients was optimal to follow the modulations of sICI across conditions. At rest sICI was weaker in ALS patients than in HS. Interestingly, we found that sICI was not modulated by the tonic contraction in ALS patients while in HS the contraction depressed the sICI. These finding suggest a dysfunction of inhibitory interneurons afferent to UMN, at early stage of ALS. This opens avenues to new approaches to counteract the imbalance of E/I observed in the motor cortex in attempt to slow down UMN loss in ALS.3 4

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353. NERVE EXCITABILITY DISENTANGLED: HYPEREXCITABILITY IN ALS IS DRIVEN BY ALTERED SLOW POTASSIUM CHANNEL KINETICS

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Background:

Changes in excitability of motor axons in MND are suggested to represent an early step in the neurodegenerative cascade. Ion-channel dysfunction is believed to play a crucial role in the observed excitability patterns. In this study, we utilize a novel approach to better disentangle the biophysical origins of motor nerve excitability changes in patients with ALS, and examine their association with several clinical characteristics.

Methods:

We prospectively recruited 167 MND patients during their first diagnostic workup and 37 age-gender matched healthy controls. Participants underwent standardized nerve excitability tests on the median nerve and thenar muscles. Clinical reference measures included: ALSFRS-R scores, survival, C9orf72 mutation status, onset region, presence of fasciculations and motor unit number estimates (MUNE) of the examined muscle. Nerve excitability recordings were age-gender corrected and z-transformed with respect to the healthy controls, after which principal components (PCs) were derived. These PCs leverage the correlations between measurement points. Associations between PCs and clinical measures were established. Then, we simulated changes in PCs to obtain novel excitability curves on which we fitted a well-established nerve model to generate insight into the origin of the excitability changes.

Results:

We retained 4 PCs explaining 64% of the variance in the excitability measures (26%, 18%, 11%, 9%, resp.). Modelling indicated that PC1 was mainly associated with voltage-gated independent properties, including resting membrane potential. Under prevailing MUNE reduction, changes in PC1 indicated decreased excitability. PC2 had the strongest relation with slow potassium gating kinetics. In contrast, reduced MUNE was associated with decreased PC2, indicative of increasing excitability. Increased PC2 was also associated with shorter survival (HR [95%CI]=1.06 [1.02-1.10], $p<0.01$) and faster decrease in ALSFRS-R at follow-up ($p<0.01$), while C9orf72 patients had lower PC2 values ($p<0.05$). PC3 was indicative of axonal refractoriness. Despite absence of clear neural origin, this value was increased patients with C9orf72 mutations ($p<0.05$), bulbar onset ($p<0.05$) and fasciculations ($p<0.01$). PC4 was best explained by sodium channel properties and ion-concentrations, but was not associated with any clinical measure.

Discussion:

We provide in vivo evidence that increased and decreased excitability are processes that can o

354. Social Cognition Impairment in Amyotrophic Lateral Sclerosis

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Introduction: Cognitive changes in patients with amyotrophic lateral sclerosis (ALS) may present as deficits in social cognition (SC). The aim of this research was to examine social cognitive abilities in healthy controls and patients with ALS by utilizing three distinct evaluations, and to determine whether there were any variations in results. This study also investigated differences in SC between ALS patients with bulbar (ALS-B) and spinal onset (ALS-S), and association of the degree of severity of motor impairment to SC impairment.

Methods: Patients with ALS (n=30) and age, sex and education matched controls (n=29) underwent a SC assessment through the Social Cognition scale on Edinburgh Cognitive and Behavioral ALS Screen (SC-ECAS), the Reading the Mind in the Eyes Test (RMET), and the Edinburgh Social Cognition Test (ESCoT).

Results: ALS patients showed significantly worse performance compared to controls in SC-ECAS (p=.024) and ESCoT (p=<.001). Despite patients reaching lower average score on RMET than controls, there were no significant differences between groups (p=.226).

We found no correlation between severity of ALS based on Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) and social cognition impairment. ALS-B showed significantly worse performance compared to patients with ALS-S on RMET (t(7.74)=-2.92, p=.021), and on ESCoT (t(9.36)=-2.79, p=.020). Despite ALS-B reaching lower average score on SC-ECAS than ALS-S, there were no significant differences between groups (p=.270).

Conclusions: Our study indicates that ALS patients show impairment in SC performance. However, there were no significant differences between controls and ALS patients on RMET. Our findings did not reveal any indication that greater levels of motor impairment in ALS cases are linked to more severe SC deficits. We have confirmed a greater SC impairment in ALS-B compared to ALS-S; yet, we found no significant differences on SC-ECAS.

355. Motor band sign is a specific marker of ALS and corresponds topographically to motor symptoms

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Background: A hallmark feature of ALS is the simultaneous involvement of upper motor neurons and lower motor neurons; evidence of both is a prerequisite for the diagnosis. There are few methods of detecting upper motor neuron dysfunction, which contributes to diagnostic delays. Meanwhile, brain MRI can demonstrate neurodegenerative iron accumulation in the motor cortex, known as the motor band sign (MBS).

Aim: To evaluate the sensitivity and specificity of the MBS for ALS and its correlation to focal motor weakness using a novel visual rating scale.

Methods: This prospective study consecutively included 117 ALS patients (66.3±12.3 years, 66 males), 79 ALS mimics (62.5±15.8 years, 46 males) and 31 age- and sex-matched neurologically healthy controls (63±14.3 years, 10 males). A 3 Tesla Siemens PrismaFit scanner with a 64-channel head coil was used to perform 3D susceptibility-weighted imaging. Three raters with varying experience (resident in radiology, fellow in neuroradiology and neuroradiologist) assessed susceptibility in the motor cortex based on a novel visual rating scale. Total and regional (medial, lateral and hand knob) MBS scores were calculated and compared between groups. Associations to the revised ALS functional rating scale (ALSFRS-R) and its subscores for fine motor, gross motor, bulbar, and respiratory function were assessed using regression analysis.

Results: Positive MBS was seen in 69 ALS patients (59%) compared to in 1 control (3.2%) and 7 ALS mimics (8.9%). This translates to a sensitivity of 59% and a specificity of 90% vs. mimics and 97% vs. controls. Higher total MBS scores were significantly associated with lower total ALSFRS-R scores (std. β=-0.30, P=0.018) but not with progression rate (P=0.64). Topographically, there was a strong association between medial MBS and gross motor dysfunction (std. β=-0.58, P=0.002), between hand knob MBS and fine motor dysfunction (std. β-0.69, P=0.007) and between lateral MBS and bulbar symptoms (std. β=-0.62, P<0.001).

Conclusions: Primary motor cortex susceptibility has high specificity but relatively low sensitivity for identifying ALS. Regional MBS scores are associated with focal motor weakness, corresponding topographically to the somatotopic organization of the primary motor cortex. These findings suggest that the MBS may be a diagnostic imaging biomarker for ALS. Its predictive value and potential role for clinical trials and treatment monitoring remains to be studied.

356. Investigating cognitive endophenotypes and presymptomatic cognition in unaffected relatives of familial ALS patients: a longitudinal study

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Background: C9orf72 is the most common genetic cause of familial-ALS (fALS). It is associated with cognitive and behavioural symptoms in ALS patients. More recently, candidate cognitive endophenotypes have been identified in unaffected fALS relatives[1], independent of C9orf72-gene status. This longitudinal study aims to assess 1) the stability of the candidate cognitive endophenotype & 2) examine longitudinal cognitive performance in unaffected fALS relatives, including C9orf72-gene carriers.

Methods: A preliminary sample of 22 unaffected first- and second-degree relatives of fALS patients (10 C9orf72-positive) and 26 healthy controls completed the ECAS and FAS phonemic verbal fluency task at two time points, 4 years apart.

Results: Groups were matched for sex ($p=0.56$) and education ($p=0.4$) but not age ($p<0.001$) or premorbid IQ ($p<0.001$). Relatives scored significantly lower than controls on ECAS Total ($b=-6.37$, $p=0.01$) and ECAS ALS-Specific domain ($b=-4.242$, $p=0.04$). This effect remained when controlling for premorbid IQ, age and education. Although relatives trended towards having lower scores on the FAS verbal fluency task, there was no main effect of group in this smaller sample ($b=-0.314$, $p=0.39$). There was a significant main effect of time on ECAS Total score ($p=0.02$) and FAS z-score ($p=0.01$), with both groups improving. However, there was no significant interaction effects of group and time for any variable ($p>0.05$). C9orf72-gene carriers ($n=10$) did not differ from non-carriers ($n=11$) on any domain ($p>0.05$).

Conclusion: Unaffected fALS relatives perform worse than controls on the ECAS, especially in ALS-Specific domains, and this is consistent over time. C9orf72-status had no bearing on cognitive performance in unaffected fALS relatives. These preliminary findings support previous work pointing to a cognitive endophenotype in kindreds of fALS patients, which may suggest an increased disease liability in this cohort.

1Costello et al.,2018

357. Cortical network dysfunction in ALS using task-free magnetoencephalography

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ALS involves complex primary pathological and compensatory dysfunction of cerebral networks. Callosal (inter-hemispheric pathway) pathology has been a consistent observation in histological and MRI studies in ALS. Magnetoencephalography (MEG) is a brain imaging technique which non-invasively measures the micro-magnetic fields generated by oscillatory brain activity. Modern computational techniques allow analysis of this data to provide highly temporally and spatially localised information about cortical neurophysiology. Task-free, resting-state MEG offers unique insight into cerebral pathophysiology by revealing dynamic, network-level changes in oscillatory brain power and connectivity.

Thirty-six non-demented patients with apparently sporadic ALS and 51 age- and gender-matched controls underwent an 8-minute resting-state MEG recording and structural MRI scan. We calculated oscillatory cortical power, connectivity and complexity measures in 52 regions and 5 canonical frequency bands (δ, θ, α, β, γ). We used maximum statistic permutations tests to look for differences between groups, and the effect of disability (ALSFRS) on brain function, correcting for age, gender and handedness.

ALS patients showed decreased beta power in sensorimotor regions (p=0.034) and increased gamma power in frontotemporal regions (p=0.015). Frontotemporal activity complexity was also increased in ALS patients (p=0.008). Higher levels of ALS patient disability were associated with increased power in these same regions (p=0.007) and increased frontotemporal connectivity overall (p=0.005) but relatively more with the contralateral hemisphere (p=0.034).

A fall and regional shift of sensorimotor beta power is the consistent underpinning of ALS cerebral pathophysiology. Increased power, connectivity and complexity of brain activity in frontotemporal regions may reflect a distinct process within ALS and FTD's pathological spectrum, or compensatory response to primary motor system disintegration. Altered functional laterality may reflect specific damage to inter-hemispheric fibres of the corpus callosum. MEG may offer a biomarker for disease progression in ALS and secondary outcome measure in therapeutic trials. The extent to which MEG network changes occur pre-symptomatically requires dedicated exploration in carriers of pathological genetic variants.

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