9TH ALS AND MND RESEARCH MEETING

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PRESENTATION SUMMARIES

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EDITORIAL

Organized every year in mid-October by the FILSLAN rare disease network in partnership with ARSLA, the Research Meeting is a key event in ALS and MND research. They unite the objectives of sharing and updating scientific knowledges on the theme of motor neurone diseases.

Last year's event attracted 150 participants, 24 oral presentations and 24 posters, demonstrating the growing dynamism of research.

This 9th edition will be marked by an international dimension with a research meeting in English exclusively. This year, 26 oral presentations and 20 posters will be presented, providing a stimulating opportunity for young researchers to share the progress of their work. It is clear that the field of ALS research is being enriched by research into other neurodegenerative diseases such as Alzheimer's. A conference on this theme will be organized. The round table will provide an opportunity to discuss artificial intelligence and its contribution to ALS research.

Pr P.Couratier

SUMMARY

FILSLAN « TRAINING THROUGH RESEARCH » PROJECT AWARD 2022

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 the misfolded prion-like Tau and a-Synuclein 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-associated USP19ΔTM or its ER-associated but 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 and immunogold electron microscopy revealed that K263E-TDP-43 was mainly released as free aggregates.

Interestingly, a significant increase of lipidated-LC3 was observed in the USP19-WT co-expressing cells. Inhibition of fusion between autophagosomes and lysosomes or inactivation of lysosomal activity using chloroquine or Bafilomycin-A1 inhibitors failed to impair K263E-TDP-43 aggregates release. Conversely, inhibition of Vps34 PI3-Kinase inhibitors strongly impaired their released release thus suggesting the essential role of the autophagosome compartments in this secretion. Surprisingly, more recently, we also observed a strong accumulation of ER compartments upon USP19-WT overexpression and identified, using an immunoprecipitation approach, physical interaction between USP19-WT and K263E-TDP-43. Presence of TDP-43 positive ER-structures in autophagic compartments suggesting an ER-phagy process involved in the release of TDP-43 aggregates mediated by USP19 is currently under investigation and will be discussed.

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SESSION 1: PATHOPHYSIOLOGY OF MOTOR NEURONE DISEASES

Moderation: Gwendal LE MASSON and Frédérique RENE

- **Guest lecture**

C1 Conference : Glial cells at the neuromuscular junction : a new therapeutic target in ALS

Richard ROBITAILLE
University of Montreal

- **Presentations selected from abstracts submitted**

OC 1.1 – Deciphering the role of TBK1 in mouse motor neurons and microglial cells, and its implications for ALS/FTD pathogenesis

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Dominant loss-of-function mutations in the ubiquitously expressed *TANK-Binding Kinase 1* gene (*TBK1*) are linked to ALS and/or Fronto-Temporal Dementia (FTD). TBK1 is involved in autophagy and innate immunity, suggesting both cell-autonomous (autophagy deregulation in motor neurons, MN) and non-cell-autonomous (altered microglial response) disease mechanisms. To decipher the role of *TBK1* in these cells, we have generated mice with *Tbk1* deletion in spinal MN or microglia. MNs were surprisingly resistant to *Tbk1* loss, and despite presence of p62+ inclusions throughout life, no loss of spinal MN was detected. In contrast, we found that *Tbk1* deletion in microglia (*Tbk1*-MG-KO) had a major impact, as it led to increased number of spinal microglia in both young and aged mice. Thus, we assessed if this modified microglial homeostasis could deregulate microglial reactive response, and by that affect MN degeneration/regeneration. First, we crossed *Tbk1*-MG-KO with $SOD1^{G93A}$ mice. Unexpectedly, *Tbk1* deletion in microglia had no impact on survival, but tended to delay symptom appearance. Next, we performed sciatic nerve crushes on *Tbk1*-MG-KO mice, and found that *Tbk1*-deficient microglia did not alter spinal MN regeneration. However, both are aggressive MN degeneration/injury models, and might override microglial dysfunctions. Thus, to understand how *Tbk1* deletion affects microglial responses, we cultured *Tbk1*-deleted microglia and found decreased responses to pro-inflammatory stimuli, emphasizing Tbk1's importance in microglia. Moving *in vivo*, we performed RNAseq on FAC-sorted microglia from young and aged mice, and found that *Tbk1* deletion led to a surprising shift of the microglial profile towards an aged phenotype. With the known microglial function in synaptic homeostasis, and implication of *Tbk1* in ALS and/or FTD, we are now assessing if the increased microglial numbers and their prematurely aged phenotype could lead to FTD-like social behavioral defects in our mice. This project could reveal how microglial deregulations contribute to, or influence ALS/FTD-linked neurodegeneration.

Mots clés : Neuroinflammation, Microglia, TBK1

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OC 1.2 - Specific role of neuronal versus microglial P2X4 receptor in ALS pathogenesis and its potential use as a biomarker

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the motor neurons (MN) loss, leading to progressive paralysis which causes the death within 3-5 years. The aggregation of misfolded proteins such as SOD1 has been associated with degeneration and high concentrations of extracellular ATP. ATP released from degenerating MNs will bind and activate P2X receptors. Among these, P2X4 receptor, which is a non-selective cationic channel has been recently involved in ALS using SOD1G93A ALS mouse model (SOD1).

We have previously found a surface increase of P2X4 in peripheral macrophages of SOD1 mice even before the symptom's onset. This result may position P2X4 as a biomarker to help diagnosis of ALS. In this study, we employed flow cytometry to measure the surface-to-total ratio of P2X4 receptors in monocytes from peripheral blood of ALS patients. Our preliminary findings indicate that there is an elevated surface density of P2X4 receptors in ALS patient as well as a potential sexual dimorphism.

To evaluate the impact of P2X4 modulation in ALS, we generated double transgenic SOD1 mice expressing either P2X4 internalization-defective knockin gene (SOD1:P2X4KI) or lacking the P2X4 gene (SOD1:P2X4KO). Surprisingly, both genotypes resulted in improved ALS symptoms, pointing out a complex cell-specific function of P2X4. To address the neuroglial role of P2X4 in ALS, we have now developed SOD1 mice, expressing either P2X4KI or P2X4KO, selectively in macrophage/microglia or neurons. Our results show cell-specific roles of P2X4 in ALS pathogenesis. The neuronal absence or the surface increase in microglia of P2X4 have both a beneficial effect on motor performance and survival of SOD1 mice which is associated with a higher survival of MNs at later disease stages.

This work may provide valuable insights into the cellular role of P2X4 to fight this fatal disease, but also defines P2X4 as a promising ALS biomarker.

Keywords:

P2X4 receptor, neuroimmune interactions, biomarker

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Financement ARSLA

OC 1.3 - Lateral hypothalamus-dependent 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 novel electroencephalography coupled with indirect calorimetry recordings to characterize sleep and energy metabolism in two mouse models of ALS -Superoxide Dismutase 1 G86R ($Sod1^{G86R}$) and Fused in Sarcoma ($Fus^{\Delta NLS}$).

In both $Sod1^{G86R}$ and $Fus^{\Delta NLS}$ 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.

Concomitantly, a sleep study was achieved in early ALS patients and presymptomatic gene carriers, with sex- and age-matched healthy controls, all devoid of respiratory defects. Using machine learning, we showed profound sleep alterations in both their sleep macro- and micro-architecture, most specifically with increased wakefulness and decreased NREM.

Thus, our results show that both ALS patients and presymptomatic gene carriers exhibit deep alterations in their sleep architecture, which were also observed in two mouse models of ALS. Our repurposed drug provided pharmacological evidence for the involvement of orexinergic neurons and a novel therapeutic approach which rescued those sleep alterations.

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Keywords: Sleep, Hypothalamus, Metabolism

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OC 1.4 - Engineered TDP43 condensates with controlled assembly/disassembly as model for studying ALS pathological aggregates in human cells

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of upper and lower motor neurons leading to muscle paralysis and death by respiratory failure 2 to 5 years after diagnosis. The disease is mainly sporadic but 10% of ALS cases are familial and no curative treatment has yet been developed. Despite the vast heterogeneity of the disease, 97% of ALS patients (sporadic & familial) exhibit mislocalization and aggregation of TDP43 in the cytoplasm of motor neurons. TDP43, encoded by the TARDBP gene, is a nuclear DNA/RNA-binding protein known to participate in various neuronal RNA metabolism activities through the formation of membrane-less organelles by liquid-liquid phase separation (LLPS). Several ALS-related mutations have been associated with an irreversible transition from these liquid compartments to a solid cytoplasmic aggregated state. However, it is still unclear whether these cytoplasmic inclusions are directly involved in cytotoxicity processes. Indeed, only a correlation, rather than a causation, has been demonstrated between the development of the disease and the presence of aggregates.
To decipher the mechanisms of the aggregation-associated neurodegeneration, we developed a method allowing the controlled assembly/disassembly of TDP43 condensates (ArtiTDP43) in human cells. ArtiTDP43 forms cytoplasmic condensates in HeLa and SH-SY5Y cells that recapitulate some of the major features of TDP43 proteinopathy. Moreover, we are currently investigating how TDP43 condensates interact with stress granules factors. Finally, preliminary data in iPSCs-derived motor neurons suggest that our method could allow the manipulation of TDP43 condensates in ALS-relevant cells. This approach might offer a promising tool to investigate the possible causal link between TDP43 inclusion formation and cellular toxicity.

Key words: TDP43, Liquid-liquid phase separation (LLPS), artificial condensates.
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OC 1.5 - Cortical sensorimotor integration in patients with ALS

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It is now well admitted that ALS affects non-motor systems. In line with this, it has been shown in SOD1 mice that neuromuscular spindles are disorganized long before motor symptoms appear, and that spindle sensory group Ia/II begin to degenerate at the same time as the first motor denervation at peripheral level. This finding indicates that sensory fibers of spindle origin are as susceptible as motor fibers to degeneration in ALS but with slower kinetics [1]. This sensory damage most likely contributes to motor degeneration by disrupting motor network homeostasis. In humans, somatosensory evoked potentials (SEPs) are impaired, likely more than currently admitted in clinics (80% of the patients we studied in [2]). Moreover, based on clinical montage for SEP (C3-C4 EEG channels), we observed that

late SEPs components (> 40-ms latency) are likely more altered than earlier components (N20-N35), likely linked to degeneration in non-motor areas [3]. To further address this question, we have designed the SOMALS study (ID-RCB 2018-A00789-52, NCT 03694132; completed; 26 sporadic without clinical signs of sensory deficit vs. 26 age-sex matched controls) including combined MEG-EEG recordings conditioned by electrical stimulation of ulnar nerve (no clinical sign of motor denervation on the side stimulated) adjusted at 1.5, 3, 6 and 9 times the perceptual threshold (xPT). All individuals had T1 MRI for valuable MEG-EEG source analysis. The preliminary results revealed that cortical activity after ulnar nerve stimulation is depressed in ALS compared to controls which supports that sensorimotor integration are altered in ALS. Further analyses are in running to compare the level of depressed activity in the different regions of interest, to explore the input/output relationship in brain cortex, and to test the possible link with clinical evaluation.

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Keywords: brain cortex, sensorimotor areas, motor network homeostasis

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OC 1.6 - Better Diagnosing and Understanding C9orf72 Repeat Instability in C9orf72-ALS Blood and Brain

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The most frequent genetic forms of amyotrophic lateral sclerosis (ALS) are linked to expanded G_4C_2 hexanucleotide repeats in the C9orf72 (C9) gene. The expanded G_4C_2 repeats are highly unstable, both intergenerationnally and intraindividually between tissues, organs and cells. It is challenging to diagnose C9 repeat size in blood and to understand the mechanisms of repeat instability in brain.

To address these challenges, we set up a novel technique of optical C9 repeat sizing (Bionano) and evaluated its precision using C9 BAC plasmids, IPSC lines and lymphoblastoid cell lines (LCL). We found

that optical repeat sizing correctly determined repeat size in BAC plasmids carrying 4, 55 or 857 repeats and the size of normal and pathologically expanded C9 alleles in IPSC and LCL.

To evaluate the diagnostic potential of optical C9 repeat sizing, we analyzed blood samples from ten C9 mutation carriers. We found that optical sizing was able to resolve the full spectrum of pathological C9 alleles including short ones of 37 repeats and long ones exceeding 6.000 repeats.

To begin to understand the mechanisms of C9 repeat instability in brain, we generated human C9 IPSC from C9 ALS patients [1] and differentiated them into forebrain cerebral organoids (COs) [2]. Immunohistochemical analysis at 64 days indicated the presence of SOX2+ pluripotent stem cells, Nestin+ neural stem cells as well as TUBB3+ and MAP2+ neurons. Optical repeat sizing demonstrated that C9 COs contain a wide range of repeat expansions and contractions, mimicking the situation in adult human brain. By contrast, the C9 IPSC lines displayed only a single pathological C9 allele. Taken together, these data suggest that C9 repeat instability starts during early neural development.

In conclusion, optical C9 repeat sizing may lay the ground for improved diagnosis of C9-linked ALS/FTD and for mechanistic studies on C9 repeat instability.

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Keywords: C9orf72, repeat instability, cerebral organoid

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OC 1.7 - One-carbon metabolism contribution to development and degeneration in ALS

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With a few exceptions, the vast majority of neurodegenerative diseases (NDD) manifests during adulthood. However, NDD onset follows a long prodromal phase, and genetic cases exist that display juvenile onset. These have contributed to the emergence of possible neurodevelopmental roots of at least subsets of NDD cases [1]. While seemingly different, neurodevelopmental and NDD share common mechanisms, including the one-carbon metabolism (1Cmet), 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 [2].

My Ph.D. project aims at investigating whether ALS might arise from neurodevelopmental impairments, focusing on the population of corticospinal neurons (CSN) and on 1Cmet. We formerly demonstrated that developmental absence of CSN delays disease onset and extends survival in the *Sod1*^{G86R} mice [3]. We hence hypothesize that alterations in 1Cmet could impact the development of CSN and contribute to disease onset later in life. To test this hypothesis, 1Cmet will be characterized and manipulated in the developing CSN of mouse models of ALS.

RNAscope allowed us to unravel opposite expression patterns of *Dhfr* and *Mthfr* in the developing cerebral cortex, with *Dhfr* mostly expressed by progenitors, and *Mthfr* by post-mitotic neurons. Additionally, we observed a significant increase in *Dhfr* expression in cortical progenitors from *Sod1*^{G86R} mice compared to controls. RNAseq data from CSN purified from *Sod1*^{G86} mice further revealed a downregulation of *Mthfr* and an upregulation of *Ahcy* during presymptomatic and symptomatic ages. Work is ongoing to determine when alterations of 1Cmet occur, and what are the consequences on brain development, disease onset and progression.

Because 1Cmet also strongly relies on diet, my project may not only inform on the consequences of ALS-related mutations, but also on the impact of developmental dietary restrictions on the potential onset of sporadic ALS.

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Key words: One-carbon metabolism, neurodevelopment, neurodegeneration

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SESSION 2: BIOMARKERS AND THERAPIES IN MOTOR NEURONE DISEASES

Moderation: Emilien BERNARD and Marie-Hélène SORIANI

- **Guest lecture**

C2 Conference: New treatments for ALS in 2023

Gaëlle BRUNETEAU
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- **Presentations selected from abstracts submitted**

OC 2.1 - Characterization of a therapeutic approach to deliver scFv targeting TDP-43 pathology in ALS

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A major hallmark of ALS is the presence of cytoplasmic aggregates of the TAR DNA/RNA binding protein (TDP-43) in 97% of patients, making this protein a major therapeutic target. The aim of our study is to develop biotherapeutics targeting TDP-43 pathology.

We previously identified single chain variable fragment (scFv) clones exhibiting TDP-43-specific affinity. The interaction of the two scFv with TDP-43 was confirmed using ELISA and Surface Plasmon Resonance (K_D=3.1E-9). We tested the effect of the scFv on TDP-43 proteinopathy in cell lines overexpressing TDP-43. One scFv (D7) decreased the level of the insoluble 35 kDa C-terminal fragment of TDP-43. Another scFv (B1) decreased the TDP-43-associated activation of NF-κB. Both scFv seemed to reverse some TDP-43-induced metabolic alterations, particularly linked to lipid metabolism.

To enhance the scFv's delivery to target cells, we complexed them to PEGylated superparamagnetic iron oxide nanoparticles (SPION). Different mass ratios of SPION to scFv were tested for their size, zeta potential, and scFv retention capacity. MTT reduction assay on HEK293T cells revealed the non-toxicity of the formulations following 4 and 24 hours of treatment. Flow cytometry and western blot analyses confirmed the cellular internalization of the SPIONs and scFv, respectively. Interestingly, there was a 30-fold increase in the internalization of the scFv when complexed to SPIONs compared to the scFv delivered using a commercial protein delivery vehicle.

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

Key words: ALS, TDP-43, scFv.

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Financement ARSLA

OC 2.2 - 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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Profiling ALS pathomechanisms could assist with the development of new treatment avenues. In this study, we aimed to validate molecular candidates with potential implication in ALS pathology which were derived from multiomic profiling studies in ALS-affected brains. Target identification was conducted in the context of the MAXOMOD consortium (*Multiomic analysis of axono-synaptic degeneration in motoneuron disease*). Multiomic analysis of human postmortem prefrontal cortex (PFC) and PFC from transgenic ALS mouse models (SOD1, TDP-43, FUS, C9Orf72) revealed multiple deregulated molecular targets and pathways. 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 cell survival. To validate selected molecular targets, we established primary cortical cultures from P0 C57/Bl6 mice. To assess the role of the targets on neuronal survival we used *in vitro* toxicity models mimicking known disease pathways in ALS, such as glutamate excitotoxicity and arsenite-induced stress granule formation. We modulated the expression of XPO-1 and MAP2K2 with pharmacological small molecule inhibitors (selinexor and trametinib). Toxicity and functionality of the inhibitors were investigated by Western blot, while neuroprotective effects of target inhibition were investigated by immunocytochemistry (cleaved caspase-3) and analysis of neurite outgrowth using image J. Our results demonstrated that 72h treatment with 20nM and 200nM trametinib, completely restored elevated phospho-Erk1/2 protein which is a direct target of MAP2K2 ($n=3$; $P < 0.05$) and significantly reduced apoptosis and increased average neurite length in glutamate-intoxicated cells ($n=5$; $P < 0.0001$). 1nM and 10nM selinexor on the other hand, significantly reduced cell death in both stress induced models ($n=5$; $P < 0.05$) but didn't affect neurite lengths. Our findings suggest that XPO-1 and MAP2K2 could be auspicious drug targets to be further explored for the treatment of ALS.

Key words: Molecular target, XPO-1, MAP2K2

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OC 2.3 - Therapeutic administration of the borna virus x protein by a viral vector AAV10 in a mouse model of ALS

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Among the pathophysiological hallmarks observed in ALS, mitochondrial dysfunctions appear to be one of the earliest events, and thus might be causative for the progressive loss of motor neurons [1]. Therefore, restoring the mitochondrial functions could be a therapeutic area of interest in the development of new therapy in the context of ALS.

For this purpose, we focused on the X protein of Bornavirus. This small protein when it targets the mitochondria acts on the preservation of mitochondrial function, inhibits apoptosis, and protect neurons from the early stages of degeneration in an animal model of Parkinson's disease [2]. Moreover, increase its mitochondrial targeting by the manipulation of his N-terminal sequence (X_{A4} protein) potentiates is neuroprotective effect [3].

Recent results obtained in the laboratory have shown that the onset of the motor deficits was delayed, the motor units were preserved, and motor neurons survival was increased in $SOD1^{G93A}$ mice treated with X protein and its derived peptide PX3. However, the life expectancy of the mice did not change.

The aims of the present study were to increase the neuroprotective potential of the X protein by improving both its delivery to motor neurons using an efficient viral vector (AAV10) and its targeting to mitochondria using the modified form X_{A4}. The viral vectors AAV10-X and AAV10-X_{A4} were injected intra-cerebro-ventricularly at birth in $SOD1^{G93A}$ mice. From these experiments, we confirmed that the X protein delays motor symptoms and improves motor neurons survival. Furthermore, promising results have been obtained with the X_{A4} protein since the life expectancy of treated mice was significantly increased compared to that of control mice.

Together, we hope that these preclinical experiments will open a new way in the investigation for effective therapeutic tools to not only improve the living conditions of ALS patients, but also to increase their lifespan.

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Key worlds: $SOD1^{G93A}$ mice; mitochondria, X protein

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OC 2.4 - A better 1-year survival prognosis estimation model for amyotrophic lateral sclerosis using UMAP and regression ridge

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The progression of Amyotrophic Lateral Sclerosis (ALS) varies considerably from patient to patient, in terms of speed and areas affected. Establishing a reliable prognosis is therefore crucial to patient care and quality of life. This makes it possible to plan interventions and choose appropriate treatments and care. Machine learning methods, based on large datasets such as the PRO-ACT (Pooled Resource Open-Access ALS Clinical Trials) database [1], have been successfully used to exploit the correlations present in these data and gain a better understanding of disease progression. In this study, our aim is to provide an aid to medical diagnosis for predicting one-year survival. Using Ridge regression [2], we were able to correctly predict a patient's survival status after one year of management in 80.83% of cases. We also use the UMAP (Uniform Manifold Approximation and Projection) method [3], a dimension reduction technique that projects patient characteristics into a two-dimensional space and groups them into subgroups with similar profiles. Thanks to this projection, we can identify the survival rate of each sub-group and predict a patient's chances of survival based on their group affiliation. In addition, we can determine which characteristics have the greatest impact on a patient's prognosis. Evaluation of our model reveals a rate of good predictions ranging from 67.93% when all sub-groups are taken into account, to 92.75% when only the most homogeneous sub-groups are considered. This approach may help clinicians to better understand individual differences in ALS progression and to develop more personalised treatment strategies.

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Machine learning, Survival prediction, UMAP
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OC 2.5 - Quantitative brainstem in amyotrophic lateral sclerosis: implications for predicting non-invasive ventilation needs

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Introduction
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder affecting motor neurons in the brain, brainstem, and spinal cord, eventually leading to respiratory failure. Regarding the management of respiratory complications in ALS patients, noninvasive ventilation (NIV) has been shown to improve survival and quality of life. However, the lack of a reliable prognostic model to predict the necessity of initiating NIV has hindered the development of clinical practices and trials.The primary objective of this study is to assess whether brainstem volumes can predict the need for initiating non-invasive ventilation. A secondary objective is to investigate the correlation between brainstem structure volumes and respiratory and bulbar functions assessed by standardized tools.
Methods
This study involved 41 ALS patients from the Parisian cohort of the PULSE study (protocol 2013-A00969-36). Volumetric analysis of brainstem regions from T1-weighted images was performed. Clinical and spirometry assessment were collected at three time points: at baseline, then at three and six months.
Results
A nominal logistic regression model incorporating brainstem region volumes provided a prediction of the need for non-invasive ventilation at six months with 85% sensitivity and specificity. Brainstem volumes were significantly correlated with the ALSFRS-R bulbar sub-score and expiratory and inspiratory functions assessed by respiratory volumes of the spirometry and the 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.
Acknowledgment
We would like to express our gratitude to all the participants and their families for their cooperation. We extend our thanks to all the experts, doctors, engineers, and technicians from the 16 centers involved in the PULSE study. The authors are grateful for the financial support from the French association ARSLA, the support from the French clinical research network FILSLAN, and the Clinical Research Federation of Lille University Hospital (CHU de Lille).

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Financement ARSLA

OC 2.6 - Multimodal automated prediction from quantitative MRI for patient classification and stratification

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The early diagnosis of patients suffering from amyotrophic lateral sclerosis (ALS) remains a difficult problem, especially concerning the prediction of faster versus slower disease progression. Solving this challenge currently faced by the clinical research community would greatly help personalizing the treatments and monitoring of patients [Paganoni *Amyotroph Lateral Scler Frontotemporal Degener* 2014, Westeneng *Lancet Neurol* 2018].

Our project aims to develop and test automated prediction tools for the stratification of ALS patients in subtypes with slow versus fast disease progression. We use quantitative magnetic resonance imaging (MRI) in order to test markers of disease progression. In particular, we rely on sodium MRI that provide measures of metabolic dysfunctions that may occur before cell death, thus

likely to contribute to early markers [Zaaraoui *Radiology* 2012, Maarouf *Neurology* 2017, El Mendili *Am J Neuroradiol* 2022]. We test multimodal prediction pipelines that combine the different MRI modalities, aiming to identify synergistic effects between them. We also test several pipeline versions that combine the data in different manners (within modality first, or within brain regions across modalities first). Finally, we identify the informative regions in the different configurations in order to gain insight on brain regions whose abnormal features may be combined together to form a signature for disease progression.

Our results for a cohort of 15 patients and 15 controls from a 7T scanner show that each MRI modality yield distinct prediction powers, where quantitative MRI outperforms classical MRI. The most informative parcels include white matter parcels and subcortical regions, but also a few cortical regions. We keep on increasing our dataset to improve the predictive power and further test the sought synergistic effects.

OC 2.7 - Implication of central nervous system barrier impairment in amyotrophic lateral sclerosis: gender-related difference in patients

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Central nervous system (CNS) barrier impairment has been reported in amyotrophic lateral sclerosis (ALS), highlighting its potential significance in the disease, as pathogenesis mechanism, as a biomarker of disease outcome and finally as part of a potential drug development strategy. In this context, we aim to shed light on its involvement in the disease, by determining albumin quotient (QAlb), reflecting CNS barrier impairment, at the time of diagnosis of ALS in a large cohort of patients. Patients from the university hospital of Tours (n=307) were included in this monocentric, retrospective study. Male and female subjects were analysed separately. Correlations between QAlb levels and parameters of the disease were evaluated. Then, we performed Cox regression to determine the association of CNS barrier impairment with survival. Nighty-two patients (30%) had elevated QAlb levels according to age-related upper reference limit. This percentage was higher in males (43%) than in females (15%). Interestingly, QAlb was not associated with age of onset, age at sampling or diagnostic delay, whether in males or females. However, we found an association with ALSFRS-r at diagnosis but this was significant only in males. QAlb levels were not linked to the presence of a pathogenic mutation. Finally, we performed a survival analysis and Kaplan-Meier curves displayed the association with survival in males only. Using the multivariate Cox proportional-hazards model, QAlb remains significantly associated with survival in male patients (HR = 2.3, 95% CI = 1.2-4.3, p=0.009). A longitudinal evaluation of markers of barrier impairment throughout the course of ALS, in combination with inflammatory biomarkers could give insight into the involvement of CNS barrier impairment in the pathogenesis of the disease. Gender difference might guide the development of new drugs and help personalise the treatment of ALS.

Mots clés : albumin quotient; central nervous system barrier; *C9orf72*;

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ARSLA SESSION

Organisation/Moderation: Cedric RAOUL and Pierre-François PRADAT (Chairmen of the ARSLA Scientific Council)

OC A1 – Relationship between diaphragm weakness, taste and smell and food intake in ALS

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Scientific background:

Amyotrophic lateral sclerosis (ALS) affects the phrenic motor neurons leading to weakness of the diaphragm. The consecutive restrictive respiratory failure is the main cause of morbidity and mortality. Non-invasive ventilation (NIV) compensates for the diaphragm weakness and corrects the associated symptoms. NIV is effective in both prolonging patients' survival and improving their quality of life. Undernutrition is another recognised prognostic factor. Weigh loss is essentially due to hypermetabolism and a reduction in food intake in bulbar involvement. In addition, diaphragmatic dysfunction directly contributes to the weight loss, as compensatory contraction of the accessory neck muscles increases resting energy expenditure. Olfactory/gustatory impairment that can be due to a decreased inspiratory capacity related to diaphragm impairment or an alteration in sensitivity recently described as part of the neurological symptoms of ALS could contribute to the weight loss in ALS.

Objectives:

1- To measure olfactory/gustatory sensitivity at various ALS stages. To correlate olfactory/gustatory sensitivity with 1/ diaphragm function, 2/ preferences for fatty, sugary and protein-rich foods, 3/ food intake and 4/ nutritional status. To investigate any central sensory involvement.

2- To investigate if increasing sensory perceptions during meal regarding patients' food preference improves the oral intake, the quality of life and the nutritional status.

Methods:

This prospective randomised open-label study enrols 50 incident patients with definite or probable ALS in two universitary hospitals in Dijon and Lyon. They undergo the following investigations over ALS course (1/ diagnosis of ALS, 2/ diagnosis of undernutrition, 3/ NIV initiation, 4/ gastrostomy):

–respiratory function: clinical signs and symptoms of weakness of the diaphragm, sitting and supine vital capacity, PaCO2, maximum inspiratory pressures (MIP and SNIP) and nocturnal SpO2

–nutritional status: BMI, muscle mass by bioelectrical impedance analysis, grip strength and resting energy expenditure (REE) by calorimetry

–eating patterns: Leeds Food Preference Questionnaire and food collection

–olfactory/gustatory sensitivity: B-SIT test (brief smell identification test), Burghart taste strips (detection thresholds for sweet, salty and umami flavours), TSQ questionnaire (Taste and Smell Questionnaire) and gustatory evoked potentials

–quality of life: ALSQoL-R (ALS-Specific Quality of Life Questionnaire-Revised) and SEIQoL-DW.

When nutritional status is impaired, patients are randomly assigned (1:1) to two groups, one with conventional dietary management, one with dietary management using sensory reinforcement during meal regarding patients' food preference.

Expected outcomes:

This work should demonstrate that diaphragmatic dysfunction has unexpected consequences on ALS patients' nutritional status. Impairment of olfactory/gustatory sensitivity might contribute to the reduction in food intake through its impact on the hedonic aspects of food intake regulation. Given the olfactory/gustatory deficit, enhancing the taste or smell of food might have a positive effect on nutritional status, quality of life and on the prognosis of ALS. Furthermore, the diaphragmatic impairment's side effect on the ingesta might be an additional argument for proposing earlier ventilatory assistance with NIV.

OC A2 – Corneal Trigeminopathy and Amyotrophic Lateral Sclerosis: Myth or Reality?

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Background: Amyotrophic lateral sclerosis (ALS) is a severe motor neuron disorder with a typical adult onset. Diagnosis is challenging due to its clinical heterogeneity and the absence of definitive diagnostic tools, leading to delays averaging between 9.1 to 27 months. In vivo corneal confocal microscopy, assessing the sub-basal nerve plexus of the cornea, has been proposed as a potential biomarker for ALS. We aimed to determine if the assessment of corneal nerves using in vivo confocal microscopy can serve as an imaging biomarker for ALS diagnosis and prognosis.

Methods: A single-center prospective case-control study was conducted in France from September 2021 to March 2023 including patients with ALS according to the revised EI Escorial criteria. Corneal sub-basal nerve plexus was analyzed using in vivo confocal microscopy. An automated algorithm (ACCMetrics) was used to evaluate the following corneal parameters: nerve fiber density (CNFD), nerve branch density (CNBD), nerve fiber length (CNFL), nerve fiber area (CNFA), nerve total branch density (CTBD), nerve fiber width (CNFW), and nerve fractal dimension (CNFractalDimension).

Results: Twenty-two patients with ALS and 30 controls were included. No significant differences were found between ALS and control groups regarding corneal innervation ($p>0.05$). Corneal sensibility did not differ between groups, and no correlation was identified between corneal nerve parameters and ALS disease duration, severity and rate of progression ($p>0.05$).

Conclusion: The present study does not support the use of in vivo corneal confocal microscopy as an early diagnostic or prognostic biomarker for ALS. Further research, especially longitudinal studies, is needed to understand any potential corneal innervation changes as ALS progresses.

Keywords: motor neuron disease; amyotrophic lateral sclerosis; visual sensory system; cornea; neuro-ophthalmology

OC A3 – Transactive response DNA-binding protein 43 is enriched at the centrosome in human cells

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The centrosome is the major organizing center of microtubules. This membraneless organelle is essential for numerous cellular processes such as polarity, genome stabilization, and ciliogenesis. Recent data indicate the presence of ribosomes and RNA-associated machinery at the centrosome, suggesting local protein synthesis. We investigated whether TDP-43, an RNA-binding protein implicated in the pathophysiology of amyotrophic lateral sclerosis and frontotemporal lobar degeneration, is localized at the centrosome. Using high-resolution subdiffraction microscopy on human cells, we found that TDP-43 is consistently present at the centrosome throughout the cell cycle. This was confirmed by Western blot and immunofluorescence assays on isolated centrosomes. Notably, TDP-43 co-localized with pericentrin, indicating pericentriolar protein enrichment. In addition, we identified four centrosomal mRNAs and 16 centrosomal proteins as direct interactors of TDP-43. Interestingly, all 16 proteins have known roles in TDP-43-associated pathophysiological conditions, suggesting the relevance of centrosomal TDP-43 in neurodegenerative diseases. Our findings mark the first identification of TDP-43 enrichment at the centrosome and lay the groundwork for further exploration of the biological functions of centrosomal TDP-43 and its role in neurodegeneration.

OC A4 – Evaluation of the potential of isoprostanoids to predict ALS progression

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Isoprostanoid quantification by LC-MS/MS currently represents a significant method, specific, and not-invasive for the lipid peroxidation evaluation, and could constitute surrogate and theragnostic biomarkers for ALS. In 2008, Mitsumoto and al., already highlighted a positive correlation between patients suffering from a sporadic ALS and the contents of 15F2t-IsoP, considered as “gold” standard of systemic oxidation. Our present study will consist of a plasma LC-MS profiling of about fifty

Isoprostanoids in patients from PULSE and healthy controls. We will validate the surrogate value of prognosis according to the ALSFRS-R rate of progression and the values of 4-HNE and 4 HHE (i.e. non specific markers of omega 3 andomega 6 peroxidation). We expect that Isoprostanoids will become surrogate biomarkers of prognosis helping stratification for clinical trials and a theragnostic biomarkers for drug acting on lipid peroxidation (i.e. deferiprone; edaravone, TUDCA, retroptope...). This presentation will focus on preliminary data obtained in the plasma assay of isoprostanoids.

Mitsumoto, H., R. M. Santella, et al. (2008). "Oxidative stress biomarkers in sporadic ALS." Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases 9(3): 177-183.

OC A5 – Development of a biomarker panel for ALS: tracking treatment efficacy in a SOD1 murine model

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To date, diagnosing ALS, tracking disease progression, and assessing treatment responses have been challenging. Identifying biomarkers observed in patients and validated in preclinical models is essential for evaluating new drugs in both preclinical and clinical trials. As ALS is a complex pathology affecting not only the nervous system but also other tissues (ie: muscles, adipose tissue) the identification of a combination of biomarkers reflecting the overall " health status" of the different tissues could enable the monitoring of disease progression and treatment efficacy. Our previous research revealed that skeletal muscles in ALS patients release vesicles harmful to motor neurons, presenting candidate biomarkers. In both ALS patients and mouse models, the metabolism affecting the management of energy stores as well as lipid metabolism are altered. In this context, monitoring muscle vesicles and their lipid content could partly reflect the altered energy metabolism in patients and could be corrected by molecules acting on this metabolism.

Hypothesis: In this project, we hypothesize that circulating muscle vesicles, alone or in combination with other biomarkers described in the literature such as neurofilaments can be used to monitor disease progression and the effectiveness of treatments for ALS.

Scientific objectives: Our project aims to validate circulating biomarkers previously identified by our lab in the serum of ALS patients, including muscle vesicles, in an ALS preclinical model, and to determine whether these markers can have predictive value for monitoring treatment efficacy.

Methodology: We first checked whether the muscle vesicle biomarkers identified in patients were also found in SOD1G86R mice and whether their levels correlated with the mice's neurological and metabolic impairments. To assess the sensitivity, we compared these biomarkers with previously identified ones. Subsequently, we aim to investigate if known therapeutic compounds can reverse

changes in these biomarkers in the mouse model, validating their clinical potential for tracking treatment effects in patients.

OC A6 – Detection of Peripherin and misfolded SOD1 as novel biomarkers in ALS

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease involving misfolding and aggregation of proteins as well as axonal degeneration. The detection of biomarkers associated with axonal degeneration or misfolding of proteins in biological fluids could provide early diagnosis. In this work we present the development of two assays. One targeting Peripherin which is neuronal type III intermediate filament expressed in spinal motoneurons and sensory neurons of the dorsal root ganglions. Peripherin, like other neurofilaments, is a potential biomarker of axonal degeneration. The second on targeting misfolded Cu/Zn superoxide dismutase 1 (SOD1) which is believed to spread with a prion-like pattern to induce relentless neurodegeneration.

We chose to use the single molecule array (SIMOA) technology which is a digital immunological sandwich assay combining high sensitivity and specificity. Clinically, this method can detect minute amounts of relevant biomarkers in biological fluids. By using antibodies designed to detect peripherin or properly folded/misfolded SOD1, we developed assays for the proteins that worked on recombinant forms. Unfortunately, the peripherin assay was not sensitive enough to detect the protein in human biological fluid. The total and misfolded SOD1 assays were however sensitive enough and we present here details of the assays' development and validation. We report sensitive differential conformational detection in both serum and cerebrospinal fluid (CSF). A small cohort of ALS' patients' CSF and serum was also analyzed with the assays. We confirmed an elevation of SOD1 in the CSF of ALS patients and we need to confirm our result on larger cohorts regarding the detection of misfolded SOD1.

SESSION 3: MOLECULAR MECHANISMS OF MOTOR NEURONE DISEASES

Moderation: Luc DUPUIS and Pascal LEBLANC

- **Guest lecture**

C3 Conference: Cryptic splicing: from foe to friend in tackling amyotrophic lateral sclerosis

Pietro FRATTA
University College London

- **Presentations selected from abstracts submitted**

OC 3.1 - The maxomod project: multiomic ALS signatures highlight sex differences, molecular subclusters and the MAPK pathway as therapeutic target

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Therapeutic options for ALS are currently insufficient, and a better characterization of early pathological changes of the disease is urgent. Here we profiled early molecular changes in ALS brains using multiomics techniques. Human postmortem prefrontal cortex tissue (PFC - affected later in the ALS pathology) of sporadic ALS patients and controls (n=51/50), as well as PFC tissue from 4 transgenic mouse models (SOD1; TDP43; C9orf72; FUS) were analyzed by multiple deep omics methods, including transcriptomics, miRNAomics (Illumina NovaSeq 6000/HiSeq 2500), and (phospho-)proteomics (label-free LC-MS (nanoLC-MS/MS)). Differential analyses were performed with custom frameworks in R, including an unbiased Multiomics Factor Analysis. Our study revealed gene and protein enrichment for multiple disease-relevant mechanisms, such as cell survival, extracellular matrix, oxidative stress/mitochondrial function, lipid and metabolism, RNA processing, synaptic function and immune response. Sex differences were observed, with more pronounced changes captured in males compare to females. Our data also showed a marked heterogeneity on the molecular level for ALS samples which could not be explained by the clinical phenotype. Furthermore, we identified that disease-associated pathways enriched for the transgenic mouse models only partially overlap with human results, indicating that the models reflect only subsets of the pathology of sporadic ALS in humans. Interestingly, we identified transcriptome-based patient subclusters that are driven by immune response, extracellular matrix, mitochondrial respiration, and RNA metabolism. We were able to demonstrate that the molecular signatures of human subclusters were reflected in specific mouse models. This Individual and integrative multiomics analysis highlighted mechanisms that were initially identified in the individual omics, but also underscored further important molecular hubs, such as the

mitogen-activated protein kinase (MAPK) pathway. Modulation of this pathway with the FDA-approved inhibitor trametinib in vitro (see poster Parvaz et al,) and in vivo validated MEK2 as an auspicious ALS therapeutic target, particularly for female ALS patients.

Key words: Multiomic, ALS-subclusters, MAPK.

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OC 3.2 - Identifying motor neuron specific alterations in fus deletion mutant zebrafish model of ALS

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FUS, mutated in ALS patients, is an RNA-binding protein, involved in multiple aspects of RNA metabolism, including RNA splicing, trafficking and translation. The majority of FUS mutations are localized in exon 15, which encodes for NLS (nuclear localization signal), causing FUS redistribution into the cytoplasm with consequent clearance from the nucleus.
Previous studies in our team reported for the first time the generation and phenotypic characterization of a stable zebrafish line mutant for the unique FUS orthologue. In this genetic line, we demonstrated that the loss of its function reduces lifespan of homozygous individuals and leads to locomotor disabilities.

Also, post-synaptic features including alterations at the mitochondrial network specifically at the muscle level were later observed in this model. Importantly, we have identified several dysregulated metabolites and proteins that are altered in heterozygotes (+/-) and homozygous (-/-) lines. From metabolomic and proteomics of whole zebrafish larvae at 3 dpf (days post fertilization) we observe important mitochondrial pathways are specifically deregulated. One of the pathways involved has been rescued by supplementing zebrafish larvae with MitoX at 2 dpf. We observe a locomotion and survival rate recovery of 40%.

In parallel to this *fus* deletion mutant model we are generating the *fus* ΔNLS zebrafish line, in order to recapitulate all the features reported for FUS-ALS pathology in patients. We will use a CRISPR technology to generate the line and subsequently validate the interact omics results observed in the *fus* KO deletion mutant in the novel zebrafish line.

These strategies will allow us to identify genetic and chemical modifiers that could rescue the phenotype due to FUS inactivation. Our objective will be to rapidly translate these findings to define therapeutics for the human pathophysiology of FUS-induced ALS.

KEY WORDS: FUS-ALS, MUSCLES MITOCHONDRIA, METABOLOMICS.
This project is funded by ANR and a 4th year thesis will be funded by ARSLA Association.
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OC 3.3 - Disrupted spontaneous neural activity in the embryonic SOD1^{G93A} mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is typically diagnosed in adulthood, suggesting a primary impact on the mature central nervous system. However, a growing body of evidence indicates that ALS onset follows a long prodromal phase, shifting the origins of the disease to early developmental stages. Interestingly, embryonic developmental stages are characterized by the presence of spontaneous neural activity (SNA) leading to giant depolarizing potentials (GDPs). We know that SNA plays crucial role in various ontogenic processes, including axon path-finding decisions, regulation of synaptic strength and gene expression. In this study, we aimed at investigating the generation of the SNA during embryonic development in the SOD1^{G93A} mouse model of ALS by using the whole-cell patch clamp technique. Specifically, we focused on the critical embryonic days 13.5 (E13.5) and E14.5 to examine SNA properties in motoneurons (MNs). Comparing SOD1^{G93A} mice with littermate wild-type (WT), we observed no changes in SNA at E13.5. However, notable alterations in SNA were discovered at E14.5, including delayed activity, decreased surface area, and amplitude of giant depolarizing potentials (GDPs). Importantly, these alterations were not attributed to the persistent sodium current (INaP) which is expressed in embryonic MNs and is known to be involved in the generation of the SNA. As SNA shapes synaptic development, we looked at synaptotagmin-2 and observed a significant downregulation in SOD1^{G93A} lumbar spinal cords at E14.5, indicating a delay in the maturation of the synaptic transmission. In conclusion, our study points out a putative altered construction of the spinal motor networks in SOD1^{G93A} and highlights the importance of investigating the early stages of ALS development.

Key words: SOD1^{G93A} ALS mouse model; spontaneous neural activity (SNA); embryonic development

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Financement ARSLA

OC 3.4 – 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. A previous discovery, a transcriptome-wide association study (TWAS), on the largest genome-wide association study (GWAS) cohort to date (29,621 cases and 120,971 controls [1]), identified NUP50, a gene encoding a nuclear pore basket protein, as a novel gene associated with ALS [2]. To date, NUP50 is thus the first direct genetic link related to nucleocytoplasmic transport, a function suspected to be affected in ALS. Twelve NUP50 variants were reported in ALS patients, which

were predicted to be pathogenic by in silico analysis [2]. However, the precise molecular mechanisms by which NUP50 alteration may contribute to ALS are 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 the nuclear import of macromolecules. Thus, we hypothesized that the variants may affect the nucleocytoplasmic transport function of NUP50. So far, two classes of NUP50 variants with distinct phenotypes were investigated in a neuronal cell culture model: NUP50 variants G114D and Y156C lead to a decrease in NUP50 protein expression, while the variant R45C leads to a loss of interaction with importin-α and NUP153. Ongoing work 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, and 3) the global interactome of NUP50 via mass spectrometry. Taken together, these first results will shed light on how NUP50 alteration may confer risk for developing ALS, potentially via nucleocytoplasmic transport deficits, which are already considered to be a contributing factor to the disease.

References :

[1] W. van Rheenen *et al*., Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology, *Nat Genet* 53, 1636–1648 (2021).

[2] Megat, S., Mora, N., Sanogo, J. *et al.* Integrative genetic analysis illuminates ALS heritability and identifies risk genes. *Nat Commun* 14, 342 (2023).

Keywords : NUP50 variants, nuclear import

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Financement ARSLA

OC 3.5 - TBK1 mutant zebrafish show increased programmed cell death and dysregulation of critical pathways involved in amyotrophic lateral sclerosis (ALS)

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ALS is a neurological disease characterized by the progressive degeneration of both spinal and cortical motoneurons that leads to muscle paralysis. *TBK1* mutations potentially leading to haploinsufficiency were first described in ALS patients in 2015. This gene is linked with autophagy and inflammation, two cellular mechanisms reported to be dysregulated in ALS patients, although its functional role in the pathogenesis remains to be defined. A well conserved *tbk1* gene has been identified in zebrafish which appears as an interesting vertebrate model to investigate TBK1 significance for motoneurons function in vivo. We generated a *tbk1* knockdown zebrafish model, using antisense morpholino oligonucleotide, which presents with an early motor phenotype from 2 days post fertilization (dpf) and is associated with degeneration of motoneurons. In parallel, we generated a *tbk1* knockout (KO) zebrafish, using CRISPR-Cas9, which shows an impaired motor function from 5 dpf and an increased lethality from 9 dpf. A metabolomic analysis in both knockdown and KO models unveiled an association between *tbk1*

loss and a severe dysregulation of the nicotinamide metabolism. Of note, incubation of *tbk1* KO larvae with nicotinamide riboside was sufficient to rescue the motor behavior defects but failed to improve the survival. Furthermore, a proteomic analysis highlights extensive markers of inflammation and a dysregulation of programmed cell death pathways. Larvae treated with the inhibitor of necroptosis, necrostatin, showed an improved survival. These findings, along with an increased cell death in the CNS measured by TUNEL staining, point towards a major role of programmed cell death in the increased lethality observed in *tbk1* KO zebrafish. Overall the tbk1 knockout and knockdown zebrafish models offer a great opportunity to better understand the cascade of events leading from the loss of *tbk1* expression to the onset of motor deficits and to the development of novel therapeutic avenues for this disease.

Keywords: ALS, zebrafish, TBK1

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OC 3.6 - Heterozygous SPTLC1 p.Leu39del is a major cause of slow-progressing juvenile ALS

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Juvenile amyotrophic lateral sclerosis (JALS) is a rare and severe motor neuron disease with currently no available therapy. Only few genes have been linked to JALS such as *ALS2*, *FUS*, *SETX*, *SPG11*, *SIGMAR1*, and more recently *SPTLC1*. This gene encodes one of the sub-units of Serine palmitoyltransferase (SPT), which is the first enzyme for *de novo* sphingolipid biosynthesis. Sphingolipids are a critical class of lipids involved in various cellular processes such as cell signaling and membrane formation. Initially, *SPTLC1* was a known cause of hereditary sensory and autonomic neuropathy, type 1A. In 2021, Johnson et al [1] and Mohassel et al [2] extended the phenotype associated with this gene by reporting several mutations of *SPTLC1* in patients with juvenile ALS. Here, we report 4 additional unrelated French families with juvenile ALS caused by the *SPTLC1* p.Leu39del variant. Lipid profile of patients were analyzed using LC-MS/MS and MS/MS. We investigated the phenotypic variability and lipidic signatures of the patients. Combined with the recently reported patient harboring the same mutation, our findings suggest that p.Leu39del is a major cause of dominant slow-progressing juvenile ALS and that in *SPTLC1* should be systematically investigated during JALS genetic diagnosis.

[1] Johnson JO, Chia R, Miller DE, *et al.* Association of Variants in the SPTLC1 Gene with Juvenile Amyotrophic Lateral Sclerosis. *JAMA Neurol* 2021;**78**:1236–48.

[2] Mohassel P, Donkervoort S, Lone MA, *et al.* Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis. *Nat Med* 2021;**27**:1197–204.

KEYWORDS

juvenile amyotrophic lateral sclerosis, *SPTLC1*, sphingolipid metabolism.

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C4 CONFERENCE

Moderation: Veronique PAQUIS

Mitophagy failure in alzheimer's disease: a hope for a diagnostic application and therapeutic targeting

Mounia Chami

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ROUND TABLE: ARTIFICIAL INTELLIGENCE CONTRIBUTION IN ALS RESEARCH

Moderation: Pierre-François PRADAT and Helene BLASCO

Theme situation:

(3 X 20 minutes, questions integrated into the debate + 1 hour discussion)

- **RT1 – Modeling and predicting the progression of neurodegenerative diseases: application to clinical trial design**
  Stanley DURRLEMAN
  INRIA, ICM, PRAIRIE ; Paris

- **RT2 – Harnessing Big Data, Omics, and AI**
  Ahmad AL KHLEIFAT
  King's College London

- **RT3 – Machine-learning based on neuroimaging patterns: opportunities and challenges**
  Peter BEDE
  Trinity College, Dublin

POSTER SESSION

P1: Beneficial effect of the environmental enrichment on a transgenic mouse model of FTD-ALS (FUSΔNLS+/-)

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The Fused in Sarcoma (FUS) is ubiquitously expressed in different tissues, including the brain. FUS protein aggregation is found in amyotrophic lateral sclerosis (ALS), where its mutation leads to predominant motor alteration, and Frontotemporal Dementia (FTD) that involves behavioral and language impairments. Enriched environment (EE) housing can enhance synaptic plasticity and memory function in animal models for age-associated neurodegenerative diseases. In this study we tested the effect of EE on a transgenic FUS mouse model of FTD/ALS, with evaluations of behavioral and transcriptomic read-outs.

The heterozygous FUSΔNLS mouse model expresses a FUS truncated protein without Nuclear Localization Signal (NLS). These mice present memory dysfunctions. Mice were housed in EE (Marlau cages, n=10-12/cage) versus Standard Environment (SE, n=2/cage), for 4 months after weaning and until tests. Five month-old mice underwent actometry, the Morris Water Maze and Object in Place. A parallel cohort was raised for RNAseq experiments performed on bulk dorsal hippocampal tissue.

We found that in the MWM task, EE-housed FUS mice of both sexes showed a significantly better performance compared to FUS SE-housed ones. In the OIP task, only males significantly performed correctly, and EE-housed FUS mice were significantly better than SE-housed mice. Transcriptomic data revealed that EE had a common effect of both FUS and WT mice by activating Immediate early genes as well as *Bdnf*. By contrast, multiple pathways related to neuronal/synaptic plasticity were dysregulated by EE only in FUS mice. Several genes involved in extracellular matrix were down-regulated in FUS mice.

Our findings demonstrate a beneficial effect of EE housing on FUS mice at the behavioral level. At the transcriptomic level, EE increased neuronal activity-associated gene transcription in both genotypes, but selectively impacted neuronal/synaptic plasticity genes in the FUS hippocampi. Further studies are needed to understand how this relates to behavioral performance.

Keywords : Enriched environment, Behavior/transcirptomics, ALS.

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P2: Manipulating fus in inhibitory neurons shed light on their contribution to ALS- and FTD-like phenotypes

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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 the protein, 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 [1], ALS-like motor impairments [2] and FTD-like behavioural dysfunctions [1]. To characterise the contribution of inhibitory neurons to these phenotypes, we created a novel mouse model based on a constitutive NLS deletion selectively in GABAergic neurons. These mice displayed FTD-like social abnormalities in the absence of ALS-like motor defects. 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 and to attenuate FTD-like social abnormalities. We are now investigating whether this also postpones the appearance of ALS pathological hallmarks and motor neuron degeneration. Ultimately, with this study, we wish to comprehend the mechanisms underlying the contribution of inhibitory neurons to ALS- and FTD-like symptoms.

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[1] Sanjuan-Ruiz I., Scekic-Zahirovic J., et al. Cytoplasmic FUS triggers early behavioural alterations linked to cortical neuronal hyperactivity and inhibitory synaptic defects, Nature Communications, 2021, 12(1): 1-19.

[2] Scekic-Zahirovic J., et al. Motor neuron intrinsic and extrinsic mechanisms contribute to the pathogenesis of *FUS*-associated amyotrophic lateral sclerosis, Acta Neuropathologica, 2017, 133(6): 887-906.

Keywords: Inhibitory neurons, Fused in Sarcoma, ALS-FTD mouse models.

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P3: Contribution of neutrophils extracellular DNA Traps to amyotrophic lateral sclerosis pathogenesis

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Purpose: Today, there is strong evidence that chronic inflammation of the central nervous system (CNS) associated with dysregulation of the immune system is involve in the pathogenesis of amyotrophic lateral sclerosis (ALS) [1]. The infiltration of lymphocytes into the brain and spinal cord of ALS patients and their involvement in the pathology have been demonstrated [2]. A study of 1,030 ALS patients showed that a higher neutrophil/lymphocyte ratio was associated with a faster disease progression and had a negative effect on the survival [3]. However, the role of neutrophils in ALS has never been investigated. Here, we first study the contribution of neutrophils and neutrophil extracellular DNA traps (NETs) in the pathophysiology of ALS.

Methods: Neutrophils were characterized by flow cytometry analyses in the blood and spinal cord of $SOD1^{G93A}$ mouse model of ALS at the asymptomatic, the onset of the disease and the symptomatic stage. Immunofluorescences were performed on spinal cord sections to visualize NETs formation. Flow cytometry panel for human blood neutrophils was designed in order to compare neutrophil sub-populations in ALS patients versus healthy controls.

Results: We observed no significative differences in the frequency and phenotype of neutrophils in the blood of $SOD1^{G93A}$ mice compare to Wild Type C57BL/6 mice from the asymptomatic to the terminal stage of the disease. However, we highlighted an infiltration of neutrophils and lymphocytes in the spinal cord of $SOD1^{G93A}$ mice at the terminal stage of the disease. Immunofluorescence on spinal cord sections revealed postive cells for myeloperoxydase, a specific marker of neutrophils and NETs. Recruitment of ALS patients and healthy controls for blood neutrophils analyses is ongoing.

Conclusion: Neutrophils are part of the immune cells that infiltrate the spinal cord in the symptomatic stage of the disease.

Reference:

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Mots-clés: SLA**,** neuroinflammation, neutrophils

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P4: Dysfunction of motor circuits is key to defects in locomotor behavior in SMA mice

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Spinal muscular atrophy (SMA) is a progressive neurodegenerative disease caused by the deficiency in the SMN protein, resulted by deletion of the *SMN1* gene. The hallmarks of SMA are death of motor neurons (MNs), muscle atrophy, and impaired motor control. MNs degenerate via a cell autonomous mechanism involving the p53 pathway [1], however, the locomotor deficits cannot be explained solely by loss of MNs. We hypothesize that synaptic dysfunction within motor circuits, represents a critical bearing on SMA pathogenesis.

Using the severe SMNΔ7 SMA mouse model, we previously demonstrated that sensory-motor circuits are severely dysfunctioning [2]. We recently investigated whether the serotonergic (5HT) and dopaminergic neuromodulation, which participates in posture and locomotion, is affected in SMA. Using mouse genetics, physiological and morphological assays we found that the effect of 5HT on the monosynaptic spinal reflex is reduced by ~80% in SMA. This dysfunction is followed by reduction of 5HT synapses on vulnerable MNs. Similarly, dopaminergic innervation is also reduced in SMA MNs. We demonstrate that MNs innervating axial musculature are preferentially affected by synaptic pruning compared to those MNs innervating distal muscles, suggesting a possible cause for the proximo-distal progression of disease. The behavioral significance of the dysfunction in neuromodulation is underlined by inter-limb discoordination in SMA mice, suggesting dysregulation of motor circuits. The locomotor discoordination is ameliorated following selective genetic restoration of SMN in 5HT and/or dopaminergic neurons.

Our work underlines the significance of dysfunction in neuronal circuits in SMA that affect motor behaviors and raise the possibility that effective therapies need to consider these circuits if normal behavior is to be restored.

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Keywords :

- Spinal Muscular Atrophy

- Neuromodulation

- Synapses

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P5: Zebra fish: a rapid tool to interpret rare variants in ALS

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Amyotrophic lateral sclerosis (ALS), is a condition that leads to death within 3 years due to progressive paralysis of the four limbs, speech, swallowing, and ultimately, breathing. It is caused by the gradual death of central and peripheral motor neurons. By studying families of ALS patients, it is possible to distinguish between familial forms (10%) and sporadic forms (90%) of the disease. Five major genes have been identified, which are involved in approximately three-quarters of familial forms and 10 to 20% of sporadic forms. However, a multitude of genes is implicated in the disease. Therefore, our team conducts molecular diagnostics on a panel of 41 genes. The current challenge lies in interpreting rare variants. Classification criteria have been proposed by the ACMG (American College of Medical Genetics and Genomics). Among them, functional data are considered as strong. It is within this framework that we have developed a functional test to help the interpretation of the pathogenicity of these variants using zebrafish (*Danio rerio*). The approach involves injecting messenger RNA containing the variant to be tested into the fertilized egg cell. Overexpression of the pathogenic human variant in zebrafish embryos leads to locomotion defects as early as 2 days and abnormalities in axonal projections as well as swim bladder formation defects. These defects are not observed in control fish. Here, we propose the use of zebrafish as an original and rapid test to aid in the interpretation of rare variants in patients with ALS. We present the results for 8 variants that could be reclassified, . The variants tested were A5V, H49P, D77V, D77del, D91A, V120L, I113M, and G13A/V15G in *SOD1* gene. The results provide strong confirmation of the pathogenicity of some of these variants and allow for a reconsideration of their assigned classification. This works suggests the usefulness of this method for other applications (diseases and/or genes).

Key words : ALS, Genetic, Zebrafish

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P6: Identification of specific molecular markers of ALS vulnerable motoneurons

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Modification of electrical activity of motoneurons is a key factor in amyotrophic lateral sclerosis (ALS) disease progression. Experimental evidence revealed a motoneuron-type vulnerability in ALS, beginning with the low excitability fast fatigable (FF) motoneurons, and the high excitability slow (S) motoneurons being preserved. These observations have led to the hypothesis that the high task demand of the FF motoneurons is responsible for their highest vulnerability.

In order to answer more broadly to the role of excitability in the selective degeneration and to improve the functional characterization of motoneurons types, we used the patch-seq method on motoneurons subtypes FF and S identified by patch-clamp electrophysiology [1] [2].

We analyzed the expression of voltage gated channels from six FF motoneurons RNA banks and six S motoneurons RNA banks and the results led us to identify several potential markers of these populations (six out of forty genes involved in action potential). Among the differentially expressed genes, we selected Cacna2d3, a gene coding for $Ca_V\alpha2\delta3$, a regulatory subunit of high voltage activated calcium channels. This subunit was significantly increased in the FF motoneurons. Our preliminary data of immunofluorescence confirmed its expression in the soma and proximal dendrites of adult spinal motoneurons, as well as at the neuromuscular junction. Its functional significance in neurotransmission and firing properties of motoneurons will be addressed thanks to the Knock-Out mice Cacna2d3$^{-/-}$ as well as its impact in ALS progression.

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Keywords: Motoneuron vulnerability, Calcium channel, Electrical activity

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P7: Propagation and toxicity of super oxide dismutase in amyotrophic lateral sclerosis using human iPSC-Derived motor neuron models

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In ALS patients, disease onset often starts focally before it affects other regions and thus could spread from cell to cell to contiguous regions. One major pathological hallmark in ALS is the accumulation of misfolded proteins into motor neurons (MNs) and this might lead to the saturation of the conventional degradation pathways. In this context, we hypothesized that accumulation of misfolded proteins could be taken up by the unconventional secretory pathway called the Misfolded Associated Protein Secretion (MAPS) pathway dependent on the USP19 deubiquitinase. This pathway has been shown to help conventional pathways to deal with overwhelming amounts of misfolded proteins. Indeed, by removing ubiquitin residues from misfolded proteins, USP19 redirects proteins from degradation. This allows the formation of DNAJC5 chaperones/misfolded proteins complexes, their translocation into late endosomes, their secretion into the extracellular space and their possible uptake by surrounding cells. Analyzing whether this pathway is involved in the secretion of ALS misfolded proteins and their propagation would help understanding disease propagation. To answer this question, MNs were generated from induced pluripotent stem cells (iPSC) of 4 patients carrying mutations in the *SOD1* gene and of control subjects. We showed that SOD1 proteins were secreted by MNs and that misfolded SOD1 proteins accumulated into mutant MNs and co-localized with DNAJC5. These colocalizations were more numerous after proteasomal inhibition. To assess whether SOD1 secretion is USP19-

dependent, we have now produced lentiviral vectors allowing the modulation of USP19 expression and we are currently analyzing the consequences on SOD1 secretion. Cocultures between control and mutant MNs will also be performed to investigate the possible USP19-dependent propagation of SOD1 from MN to MN. In conclusion, this study could allow the identification of a secretory pathway involved in the propagation of ALS pathological determinants.

Keywords: iPSC, USP19, misfolded proteins.

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P8: Role of the sympathetic autonomous system in ALS

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Amyotrophic lateral sclerosis (ALS) is primarily known for its degenerative effect on motor neurons. However, additional non-motor dysfunctions, such as changes in the autonomic nervous system, have been documented in both ALS patients and animal models. Moreover, disruptions in energy homeostasis have been observed as ALS progresses, with noticeable metabolic changes occurring even before the onset of motor symptoms [1]. Engaging in motor activities requires an elevated energy expenditure, which relies on effective coordination between the sympathetic and somatic motor systems. While supraspinal structures play a crucial role in this adaptive process, we have recently shown that the activity of sympathetic preganglionic neurons (SPNs), the primary output of the sympathetic nervous system (SNS), and motoneurons (MNs) can be coupled through pharmacological activation of an intraspinal network, revealing a spinal-independent coupling between these two neuronal subtypes [2]. Moreover, MNs and SPNs share the same embryonic origin as well as numerous genetic similarities, common molecular modulators. Interestingly, SPNs innervate the connections between MNs and muscles, the neuromuscular junctions (NMJs), and play a crucial role in the function and stabilization of these synaptic connections, as well as in the cholinergic release. The sympathetic and somatic systems thus appear to be anatomically and functionally coupled at both the spinal and muscular levels [3]. Our project explores possible dysfunctions or alterations of the SNS over the progression of ALS in the SOD1 mouse model of ALS. In live animals, we have measured cardiovascular parameters of anesthetized animals in response to SNS modulation using a tail-cuff device. Furthermore, we have examined cardiovascular parameters of awake and freely moving animals before and after intense physical exercise using a telemetric approach. Transcriptomic and immunohistochemical techniques were employed to investigate potential changes in SPNs throughout the progression of ALS.

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Keywords: sympathetic autonomic nervous system, spinal cord, sympathetic preganglionic neurons

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P9: Involvement of cerebello-spinal projections in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by the loss of motor neurons in the brain and spinal cord, leading to progressive paralysis and death. In addition to the pathological processes centred around the motor neuron, cells surrounding their environment and other regions of the nervous system appear to be involved in the onset and progression of the disease. Clinical imaging studies in patients have revealed structural defects in the cerebellum.
The cerebellum integrates sensory motor information to regulate motor commands. The deep cerebellar nuclei (DCN) are the sole output relay of the cerebellum. Recently, it has been shown that DCN projects directly onto the spinal cord, and more precisely to the V1 interneurons [1]. Those interneurons seem to be preferentially lost in ALS, as they connect the most vulnerable population of fast motor neurons [2].

Our goal is to understand the role of the cerebellum and its spinal cord projections in pathology and to evaluate its potential as a therapeutic target.

The first step is to confirm the profile of V1-interneuron in ALS, since there are few studies focusing on them. For these purposes, we performed immunofluorescence on spinal cord slices with antibodies directed against Engrailed-1 and Foxp2 (expressed by V1-interneurons) and MMP9 (marker of fast fatigable motor neurons). We study the expression of those markers at various pre-symptomatic and symptomatic stages to evaluate the degenerative process.
To investigate the cerebello-spinal projection, we use an anterograde trans-synaptic virus that allows us to trace the synaptic projection of the DCN on the spinal cord. We will then identify and characterize DCN targets in the spinal cord and study the evolution of these synapses during the pathology in mice models of ALS.
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Keywords: cerebellum, interneurons, viral neuronal tracing

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P10: Association Between Brain and Upper Cervical Spinal Cord Atrophy Assessed by MRI and Disease Aggressiveness in Amyotrophic Lateral Sclerosis

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BACKGROUND AND PURPOSE

Significant brain and spinal cord atrophy was evidenced in patients with ALS, predicting progression and survival [1,2]. However, the contribution of upper (UMN) and lower motor neuron (LMN) degeneration to disease aggressiveness as well as their relationship are far from being clarified [1,2].
The objective of this study was to characterize the relative contributions of brain and upper cervical spinal cord compartmental atrophy to disease aggressiveness in ALS, using a conventional brain MRI scan.

MATERIALS AND METHODS

Twenty-nine ALS patients and 24 age- and gender-matched healthy controls (HC) were recruited. Disease duration and the ALSFRS-R at baseline, 3- and 6-months follow-up were assessed. Patients were clinically differentiated into fast (n=13) and slow (n=16) progressors according to their ALSFRS-R progression rate.
Brain grey (GM) and white matter, brainstem sub-structures volumes and spinal cord cross-sectional area (SC-CSA) at C1-C2 vertebral levels were measured from a 3D-T1-weighted MRI sequence at 3T.

RESULTS

Fast progressors showed significant GM, medulla oblongata and SC atrophy compared to HC (p<0.001, p=0.013 and p=0.008) and significant GM atrophy compared to slow progressors (p=0.008).
GM volume correlated with the ALSFRS-R progression rate (R^2/p=-0.487/0.007), the ALSFRS-R at 3-months (R^2/p=0.622/0.002), and ALSFRS-R at 6-months (R^2/p=0.407/0.039). Medulla oblongata volume and SC-CSA correlated with the ALSFRS-R at 3-months (R^2/p=0.510/0.015 and R^2/p=0.479/0.024). MRI measures showed high performance to discriminate between fast and slow progressors (Receiver operator characteristic analysis: area under curve/specificity/sensitivity/accuracy=0.764/0.813/0.692/0.759).

CONCLUSION

Our study suggests an association between compartmental atrophy and disease aggressiveness. This result is consistent with the combination of upper and lower motor neuron degeneration as the main driver of disease worsening and severity in ALS [1,3]. Our study highlights the potential of brain and spinal cord atrophy measured by MRI as biomarker of disease aggressiveness signature.

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KEYWORDS

Disease aggressiveness, MRI, brain and spinal cord atrophy

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P11: Homozygous COQ7 mutation, a new cause of potentially treatable distal hereditary motor neuropathy

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Distal hereditary motor neuropathy represents a group of motor inherited neuropathies leading to distal weakness. We report a family of two brothers and a sister affected by distal hereditary motor neuropathy in whom a homozygous variant c.3G>T (p.1Met?) was identified in the COQ7 gene. This gene encodes a protein required for Coenzyme Q10 biosynthesis, a component of the respiratory chain in mitochondria. Using patient blood sample and fibroblasts derived from a skin biopsy, we investigated the pathogenicity of the variant of unknown significance c.3G>T (p.1Met?) in the COQ7 gene and the effect of Coenzyme Q10 supplementation in vitro.

We showed that this variation leads to a severe decrease in COQ7 protein levels in the patient's fibroblasts, resulting in a decrease in Coenzyme Q10 production, and in the accumulation of 6-demethoxycoenzyme Q10, the COQ7 substrate. Interestingly, such accumulation was also found in the patient's plasma. Normal Coenzyme Q10 and 6-demethoxycoenzyme Q10 levels were restored in vitro by using the Coenzyme Q10 precursor 2,4-dihydroxybenzoic acid, thus bypassing COQ7 requirement. Coenzyme Q10 biosynthesis deficiency is known to impair mitochondrial respiratory chain. Seahorse experiments showed that the patient's cells mainly rely on glycolysis to maintain sufficient ATP production. Consistently, the replacement of glucose by galactose in the culture medium of these cells reduced their proliferation rate. Interestingly, normal proliferation was restored by Coenzyme Q10 supplementation in the culture medium, suggesting a therapeutic avenue for these patients.

Altogether, we have identified the first example of recessive distal hereditary motor neuropathy caused by a homozygous variation in the COQ7 gene. Furthermore, 6-demethoxycoenzyme Q10 accumulation in the blood can be used to confirm the pathogenic nature of the mutation. Finally, supplementation with Coenzyme Q10 or derivatives should be considered to prevent the progression of COQ7-related peripheral inherited neuropathy in diagnosed patients.

COQ7, dHMN, Coenzyme Q10

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P12: Unconventional secretion of misfolded SOD1 and toxicity spreading: a novel therapeutic strategy for amyotrophic lateral sclerosis

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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 how ALS-causing protein can be secreted and be therapeutically targeted to stop disease progression. METHODS & RESULTS: Through the description of unconventional secretion mediated by USP19, we study the secretion of $SOD1^{G93A}$ mutant using cell culture system and expression of functional mutants. The analysis of UPS19 expression pattern is done at different disease stages by immunofluorescence and biochemistry in $SOD1^{G93A}$ mice. Besides, innovative technologies are validated to silence efficiently USP19 *in vitro*. Our data show that the USP19 promotes the secretion of $SOD1^{G93A}$ 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.

Key words: prion-like, neurodegenerative disease, unconventional secretion

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P13: Metabolic reprogramming of regulatory T cells as a therapeutic strategy for amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive degeneration of motoneurons in the brain and spinal cord. Currently, no effective treatment is available, highlighting the need for novel therapeutic strategies. ALS has a complex etiology. Specifically, immune dysregulation and metabolic abnormalities contribute to disease progression.

Recent studies have demonstrated the involvement of the adaptive immune response in ALS, particularly the role of CD8+ T lymphocytes and regulatory CD4+ T cells (Tregs). While CD8+ T cells promote motoneuron death [1], Tregs have shown neuroprotective effects, by suppressing neurotoxic T cells and inflammation, prolonging survival in ALS mouse models [2]. In ALS patients, the number of

Tregs increases during the early stage of the disease but rapidly decreases as the disease progresses. Furthermore, Tregs from ALS patients exhibit reduced suppressive activity, suggesting a potential therapeutic target [3].

This research aims to investigate the metabolic status of Tregs in ALS mice and patients and explore the potential for modulating Treg metabolism to enhance their suppressive function and stability for the development of novel neuroprotective cell therapy.

To achieve this objective, the following steps will be carried out: (1) identify the metabolic and lipidomic profiles of Tregs isolated from ALS mice and patients; (2) determine optimal conditions for metabolic reprogramming of ALS Tregs and evaluate their immunosuppressive and neurotrophic properties *in vitro*; (3) investigate the therapeutic potential of reprogrammed ALS Tregs through adoptive transfer into ALS mice, assessing motor behavior, motoneuron survival, and neuroinflammation; (4) transfer reprogrammed patient Tregs into immunodeficient ALS mice to evaluate their impact on disease markers and suppressive functions.

By understanding the role of cell metabolism in regulating Treg function, this research has the potential to provide valuable insights into the pathogenesis of ALS and offer new therapeutic avenues for the treatment of this devastating disease.

Keywords: neurodegeneration; Treg; metabolism; cell therapy

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P14: Preconceived ideas about ALS’s medical knowledge: a philosophical approach

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There are many preconceived ideas about the state of medical knowledge and medical practices related to ALS: ALS is said to be an exemplary case of medical difficulties, between therapeutic powerlessness and etiological ignorance. The common characterization of ALS as "the worst disease" feed the idea that medicine is particularly ignorant and powerless in dealing with this disease. The ALS community strives to show the fallacy of these ideas by exposing the actual state of medical knowledge and practices.

To understand what these preconceived ideas are, we use a philosophical approach to identify their origins and mechanisms of persistence. This investigation is based on the study of a plurality of discourses on ALS: illness narratives, press articles, popularization articles, historical literature, ethical literature and biomedical literature. The reading of these different texts allows identifying the places where received ideas are expressed and to characterize them more precisely. We show that these ideas are mostly expressed in discourses that do not directly address the actual state of medical knowledge and medical practices related to ALS. Some of the formulas used in these speeches exacerbate the description of an exemplary medical powerlessness and ignorance in the face of ALS. Far from being mere rhetorical effects, these formulas convey deleterious preconceived ideas in terms of their epistemological and practical consequences.
We continue by arguing that the persistence of such preconceived ideas constitutes an epistemic challenge, i.e. that it results from a confusion between different perspectives on the disease. In order to challenge them and to avoid this confusion between the different dimensions of this disorder, a dialogue between these different perspectives should be encouraged.

Keywords: philosophy – preconceived ideas – epistemic challenge

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P15: Evaluation of the safety and efficacy of the atalante exoskeleton in the rehabilitation of patients with amyotrophic lateral sclerosis

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Background: Using a MRI gait motor imagery paradigm, we showed a reorganization of the motor networks that represents a compensatory response to the dysfunction of the networks involved in gait function [1]. Our main hypothesis is that by providing coherent proprioceptive input to the sensorimotor integration areas, gait training with an exoskeleton may boost compensatory network reorganization and help to maintain function.

Objectives: This study, aims to evaluate the participants subjective experience, the safety and the effectiveness of this training compared with usual care, on walking and other symptoms associated with motor disability.

Methods: The Atalante exoskeleton is the only self-stabilizing exoskeleton that enables assisted walking without walking aid, reproducing a natural walking pattern and multidirectional movement, guaranteeing therefore consistent sensory feedback. We will conduct an interventional, monocentric, prospective, open trial. We will include 20 patients with slowly progressing ALS and walking deficits. The study will be conducted in 3 phases, each lasting 8 weeks, following the ABA procedure. Phase B represents the intervention phase, during which patients will practice their gait training at a rhythm of

3 sessions/week. An evaluation is planned before, in the middle of and at the end of each phase. The main evaluation criterion is the safety of the exoskeleton. Participants' subjective experience, intrinsic motivation, gait analysis, functional status, spasticity, balance, muscle strength, quality of life (QoL), pain, anxiety and depression will constitute the secondary endpoints.

Results: The inclusion of patients is planned to start on Q3 2023. We will present the design of the protocol.

Conclusions: Large-scale implementation of the Atalante exoskeleton can improve independence and QoL in ALS patients. This study will be essential for the design of a future RCT to assess the effect of gait training using the Atalante exoskeleton on neuroplasticity and muscle neurophysiology compared with conventional rehabilitation.

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Key words: Amyotrophic lateral sclerosis, Atalante exoskeleton, gait training.

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P16: The premodials project: identification of a disease signature for presymptomatic and early ALS

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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. Although several biomarkers have been identified, including neurofilaments in serum and cerebrospinal fluid (CSF) and soluble p75ECD in urine, a multi-modal signature might be more sensitive to detecting early or even pre-symptomatic disease. 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). In this study, we aim to identify a panel of ALS biomarkers that differentiate PGMC and early ALS from controls and disease mimics.
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 early ALS or ALS mimics (n=110 per group) will be recruited and assessed longitudinally. 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 analysed by mass spectrometry and targeted immunoassays. Data will be processed by standardised protocols and consecutively integrated to identify clinical and molecular fingerprints of ALS. With this study, we aim to empower early, and even pre-symptomatic, diagnosis of ALS, and ultimately facilitate early treatment. The "PremodiALS" project now received ethical approval and recruitment started in Q1/2023.

Key words: ALS, pre-symptomatic, Biomarker**.**

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P17: 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 ALS, non-conventional MRI has shown alterations of microstructure using diffusion MRI and recently sodium homeostasis with sodium MRI [1]. 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 ALS patients and 24 age- and gender-matched healthy controls (HC) were recruited. Clinical assessments included disease duration and the 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) ^{1}H T1-weighted and diffusion sequence; (2) ^{23}Na 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, along with higher TSC and 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 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 [2]. Interestingly, fast progressors showed widespread combined alterations while slow progressors only showed restricted microstructure damage. Our study highlights the relevance of a multinuclear MR imaging approach to stratify patients according to their disease aggressiveness. Non-conventional and multiparametric MRI technics especially when combined with mathematical modeling and artificial intelligence might contribute to predict disease progression trajectory of a single patient [3].

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KEYWORDS

Disease severity, sodium imaging, diffusion tensor imaging

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P18: Functionnal analysis of genetic variants in amyotrophic lateral sclerosis by studying early markers of neurodegeneration

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20% of cases of amyotrophic lateral sclerosis are due to pathogenic genetic variants. Over 30 genes have been linked to the development of the disease to date, and the gene coding for Superoxide Dismutase 1 (SOD1) is one of them. An antisense therapeutic targeting the SOD1 gene (Tofersen) can be administered only to patients carrying pathogenic variants in this gene.

Over 200 different SOD1 gene variants have been identified in the disease, some of which are considered potentially pathogenic or of unknown significance. This is why we decided to study the

function of 20 of these particular SOD1 variants identified in ALS patients in the Biochemistry and Molecular Biology Department of the Hospital of Tours.

The different variants are created by site-directed mutagenesis on a plasmid expressing the human wild-type SOD1 protein. The plasmids obtained are used for various in vitro functional analyses using different cell types. Several markers of neurodegeneration, such as the ability to form protein aggregates and cell viability, are studied in HEK cells. The enzymatic activity of superoxide-dismutase is also studied in HEK cells. Neurite morphology (size, complexity) is studied in differentiating NSC34 cells and in primary neurons, the latter allowing studies of dendritic spine morphology. Some of the variants of interest from previous in vitro studies will then be studied in the Zebrafish model, in collaboration with a team from Montpellier (INM).

The results obtained could be exported to the medical laboratory and the clinic, with a possible return to patients if variants of unknown significance or probably pathogenic are reclassified as pathogenic variants (possibility of therapeutic intervention). In addition, the in vitro and in vivo models developed could be used for therapeutic studies under development within the team or in line with collaborators.

Keyword : SOD1, variant, function

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P19: A non-pharmacological neuromodulative therapeutic approach for amyotrophic lateral sclerosis; a systematic review

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In ALS, the benefit of physical activity and non-invasive brain stimulation remain controversial [1, 2]. The aim of this systematic review was to evaluate current research to provide a conclusive answer whether non-pharmacological therapeutic approach i.e., exercise-based therapy and non-invasive brain stimulation, produce clinical functional and neuromodulative changes in individuals with ALS. PRISMA-20 guidelines were used and the literature was searched using the following electronic databases: PubMed, CENTRAL, NIH PMC, PEDro, ScienceDirect, Web of science between January 10th 2023 and February 17th 2023. Randomized controlled trials with ALS participants were screened. The main outcome measure was the ALSFRS-R scale. The secondary outcome measures were clinical measures and electrophysiological measures. 12 studies were included in the final analysis. The internal validity of the documents was evaluated using the PEDro scale. Three studies shown an improvement of the function with exercise-based therapy but no conclusion could be drawn about their effects on neuromodulation. Regarding non-invasive brain stimulation, the results were conflicting and no conclusion could be drawn too. Many questions remain unresolved regarding the effects of non-invasive brain stimulation or exercise-based therapy. Nevertheless, exercise-based therapy seems promising to reduce functional disabilities in patients with ALS. Future randomized controlled trials should be conducted to examine their effects on neuromodulation. Non-invasive brain stimulation could be used to assess the potential neuromodulative effects of exercise-based therapy and to identify new biomarkers of neuroplasticity. PROSPERO; Reg. n°CRD42023408121.

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Keywords: Physical therapy, Stimulation, Neuromodulation

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P20: Similar changes in glycosphingolipid metabolism in ALS and viral infection are modulated by ambroxol

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Ambroxol is a generic drug, with good safety, sold in 77 countries as a mucolytic, which inhibits the non-lysosomal glucocerebrosidase GBA2, while being a chaperone of the lysosomal enzyme GBA1 [1]. Metabolomic studies had shown that GBA2 activity was specifically increased in spinal cord of presymptomatic $SOD1^{G86R}$ mice [1], increasing ceramide and depleting glucosylceramide and the neurotrophic glycosphingolipid, GM1, associated with denervation. GM1 is a major target for viruses and SARS-CoV-2. Glucosylceramidase synthase inhibitors are deleterious in ALS models, while GBA2 inhibitors are beneficial [2]. Ambroxol was also effective in $CHMP2B^{intron5}$ mice and $TDP43^{Q331K}$ mice, being the first compound to be active in the three models, having beneficial effects on denervation. A six-month phase II clinical trial has been started by Prs Vucic and Kiernan, supported by Fight MND (ALSFRS-R, time to event, MUNIX, split-hand index, glycosphingolipids (GSLs) using metabolomics). Ceramide, glycosphingolipid, sphingomyelin and phospholipid metabolism indicate actions at endoplasmic reticulum/mitochondria-associated membranes [2, 3], where viruses such as SARS-CoV-2 replicate. The metabolomic effects of ALS and SARS-CoV-2 have initiated an action by JPND to assess whether COVID/long COVID can increase risks of neurodegenerative diseases via GSLs: the conclusions will be shared at the meeting.

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Mots clefs : ambroxol, glucosylceramidase, metabolomics.
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