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Filière de Santé Maladies Rares Sclérose Latérale Amyotrophique et Maladies du Neurone Moteur

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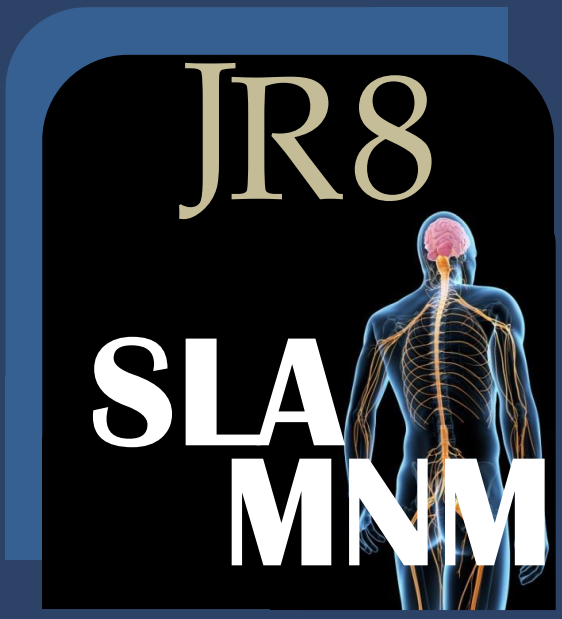
ARSLA
Association pour la Recherche sur la Sclérose Latérale Amyotrophique et autres Maladies du Motoneurone

8èmes Journées de la Recherche sur la SLA et les Maladies du Neurone Moteur

RÉSUMÉS DES PRÉSENTATIONS



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Codes de présentation : C = conférence, PO = présentation orale, TR = table ronde et P = poster

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ÉDITO

Devenues un rendez-vous incontournable des chercheurs français et francophones, les Journées de la Recherche sur la SLA et les Maladies du Neurone Moteur, organisées tous les ans par la Filière de Santé Maladies Rares FilSLAN en partenariat avec l'ARSla autour de la mi-octobre, arrivent en 2022 à leurs 8èmes édition. Elles unissent des objectifs de partage et d'actualisation des connaissances scientifiques dans le thème des maladies du neurone moteur. Après deux éditions au format virtuel, les Journées de la Recherche SLA/MNM font leur retour en présentiel dans les locaux de l'ICM à Paris.

Les Journées de la Recherche SLA/MNM réunissent environ 120 chercheurs institutionnels et cliniciens répartis dans des équipes de recherche nationales. Elles démontrent la dynamique croissante de la recherche nationale sur le thème de la SLA. Les sessions scientifiques et posters sont l'occasion stimulante pour de jeunes chercheurs de faire connaitre l'avancée de leurs travaux. Quatre communications sont récompensées par un prix ARSla après sélection par un jury émanant de son Conseil Scientifique. Les Late Breaking News, nouveauté de cette édition, seront l'occasion de balayer les dernières actualités. Force est de constater que le champ de recherche sur la SLA s'enrichit des recherches sur d'autres maladies neurodégénératives comme la maladie de Parkinson, une conférence sur ce thème est organisée. La table ronde quant à elle sera l'occasion de s'interroger sur l'avenir des ASO.

Pr P. Couratier

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C1-Conférence : Cytosquelette et transport axonal

Ludo VAN DEN BOSCH
Leuven

- **Présentations sélectionnées à partir de l'appel à communication**

PO 1.1 : CHCHD10 AND SLP2 CONTROL THE STABILITY OF THE PHB COMPLEX : A KEY FACTOR FOR MOTOR NEURON VIABILITY

Genin EC (1), Bannwarth S (1)*, Ropert B (1), Lespinasse F (1), Mauri-Crouzet A (1), Augé G (1), Fragaki K (1), Cochaud C (1), Donnarumma E (2), Lacas-Gervais S (3), Dupuis L (4), Wai T (2) and Paquis-Flucklinger V (1)*

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CHCHD10 is an amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD) gene that encodes a mitochondrial protein whose precise function is unclear. Here we show that CHCHD10 interacts with the Stomatin-Like Protein 2 (SLP2) and participates to the stability of the Prohibitin (PHB) complex in the inner mitochondrial membrane. By using patient fibroblasts and mouse models expressing the same *CHCHD10* variant (p.Ser59Leu), we show that SLP2 forms aggregates with prohibitins, found *in vivo* in the hippocampus and as aggresome-like inclusions in spinal motor neurons of $Chchd10^{S59L/+}$ mice. Affected cells and tissues display instability of the PHB complex which participates at least in part to the activation of the OMA1 cascade with OPA1 processing leading to mitochondrial fragmentation, abnormal mitochondrial cristae morphogenesis and neuronal death found in spinal cord and the hippocampus of $Chchd10^{S59L/+}$ animals. Destabilization of the PHB complex leads to the instability of the mitochondrial contact site and cristae organizing system (MICOS) complex, likely *via* the disruption of OPA1/Mitofilin interaction. Thus, SLP2/PHB aggregates and destabilization of the PHB complex are critical in the sequence of events leading to motor neuron death in $CHCHD10^{S59L}$-related disease [1]. We also observed SLP2/PHB aggresome-like inclusions in spinal motor neurons of $Fus^{\Delta NLS}$ mice suggesting that the pathological cascade associated with *CHCHD10*-related disease could be involved in other ALS models.

Reference :

[1] Genin E.C, Bannwarth S, Ropert B *et al*. CHCHD10 and SLP2 control the stability of the PHB complex: a key factor for motor neuron viability. Brain, 2022 Jun 3; awac197. doi: 10.1093/brain/awac197.
Keywords : *CHCHD10*, ALS, mitochondria
Acknowledgements : This work was made possible by grants from the ANR (Agence Nationale de la Recherche) ANR-16-CE16-0024-01 and from the FRM (Fondation pour la Recherche Médicale) FRM: MND202004011475.
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PO 1.2 : ENGRAILED-1 HOMEOPROTEIN IS A NON-CELL AUTONOMOUS NEUROTROPHIC FACTOR FOR MOTONEURONS

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In the mouse spinal cord, the homeoprotein transcription factor EN1 is expressed in V1 interneurons, including Renshaw cells, that synapse on α-motoneurons (αMNs). In midbrain neurons EN1 exerts neuroprotection and may do the same on αMNs following intercellular transfer. We thus evaluated the non-cell autonomous activity of EN1 on MN physiology and survival.
We first characterized ventral spinal neuron survival in mice heterozygous for *En1* (*En1*$^{+/-}$). Whilst the number of V1 interneurons is not modified in *En1*$^{+/-}$ mice up to 15,5 months of age, these animals display i) reduced muscle strength and an abnormal extensor reflex starting at 2 months of age, ii) neuromuscular junction (NMJ) denervation at 3 months of age and iii) significant αMN loss at 4.5 months of age, associated with an increased expression of the p62/SQTSM1 autophagy mark. Recombinant human EN1 (hEN1) injected intrathecally at lumbar level 5 of 3-month-old mice gains specific access to MNs, prevents αMN loss and restores both muscle strength and NMJ innervation. Phenotypic rescue is maintained for 3 months following a single injection and can be prolonged with a second injection. Viral expression of a secreted single chain anti-EN1 antibody (scFvEN1) by astrocytes dampens EN1 transfer into MNs in WT mice and phenocopies the deficits displayed by *En1*$^{+/-}$ animals.
Taken together, our data demonstrate that non-cell autonomous EN1 is a MN neurotrophic factor with potential interest for the treatment of MN diseases.
Keywords: Engrailed1, homeoprotein, motoneuron
Funding sources: Memolife Labex PhD fellowship (SEVA), Association Nationale de la Recherche et de la Technologie (Cifre/ANRT n°2017/0488, ML), BrainEver, Homeosign ERC-AdG n°339379.
Conflict of interest statement: AP and KLM are co-founders and hold shares in BrainEver SAS, a company developing HPs for therapeutic use.
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PO 1.3 : CHEMOGENETIC SILENCING OF CORTICOFUGAL NEURONS MAY REQUIRE FINE TUNING WHEN ASSESSING CORTICAL NETWORK DYSFUNCTION IN THE SOD1^{G86R} MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Cortical network dysfunction is a hallmark of ALS, and cortical hyperexcitability was demonstrated to precede muscle denervation, negatively correlate with patient survival, and be temporarily corrected by Riluzole in ALS patients [1]. In the $Sod1^{G93A}$ mouse model of ALS chronic activation of the cortical parvalbumin-positive interneurons, the main inhibitory output onto corticofugal neurons (including corticospinal neurons), delayed disease onset and extended the animals' life span [2]. On the other hand, chronic activation of corticofugal neurons in wildtype animals was sufficient to trigger motor symptoms and core neuropathological hallmarks of ALS [3]. Taken together, these results suggest that cortical network dysfunction may actively contribute to disease progression through its main outputs, the corticofugal tracts. To better understand the contribution of the corticospinal and other corticofugal neurons in the disease onset and progression in ALS, we chemogenetically silenced the corticofugal neuronal populations present in the motor cortex of the $Sod1^{G86R}$ mouse model of ALS using the DREADD silencing receptor hM4Di. Four groups of animals were generated ($Sod1^{G86R}$ or wildtype, injected either with hM4Di or control), and chronically treated with the DREADD ligand Clozapine-N-Oxide (CNO) in drinking water from the pre-symptomatic ages of 30 or 60 days, until disease end-stage. Animals were then longitudinally followed to record disease onset and survival, weight and motor performances. In our conditions, the corticofugal neuron silencing did not improve the survival of the $Sod1^{G86R}$ mice and had no significant effect on their motor performance. Because this negative results could arise from a possible homeostatic response of the cortical circuits to chronic silencing of the corticofugal neurons, different CNO administration approaches are currently being explored, along with alternative silencing protocols. The study intends to evaluate whether cortical hyperexcitability could represent a new therapeutic target in ALS.

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[1] Eisen, A., Braak H., Del Tredici, K., *et al.* Cortical influences drive amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*, 2017; 88: 917–924 (2017). [2] Khademullah, C. S., Aqrabawi, A. J., Place, K. M., *et al.* Cortical interneuron-mediated inhibition delays the onset of amyotrophic lateral sclerosis. *Brain : a journal of neurology,* 2020; 143: 800–810. [3] Haidar, M., Viden, A., Cuic, B., *et al.* Cortical hyperexcitability drives dying forward ALS symptoms and pathology in mice. *bioRxiv* 2021; 1–36 (). doi:10.1101/2021.08.13.456320

Keywords: cortical network dysfunction, corticofugal neurons, DREADD silencing

Acknowledgements: We are grateful to the ARSLA and the ERC for funding this project.

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PO 1.4 : 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. We have previously conducted a discovery transcriptome-wide association study (TWAS) on the largest genome-wide association study (GWAS) cohort to date (29,621 cases and 120,971 controls [1]) and identified 30 loci associated with ALS [2]. Interestingly, decreased expression of *NUP50*, a gene encoding a nuclear pore basket protein, was associated with ALS in

TWAS. To date, *NUP50* is thus the first direct genetic link related to nucleocytoplasmic transport, which is suspected to be affected in ALS. We reported twelve *NUP50* variants present in ALS patients which are predicted to be pathogenic by *in silico* analysis [2]. Importantly, knocking down *NUP50* led to increased neuronal death associated with p62 and nucleoporin inclusions in cultured neurons [2]. However, the precise molecular mechanism by which NUP50 alteration may contribute to ALS is unknown. As the *NUP50* variants identified were all located in the binding domains to importin-α and nucleoporin 153 (NUP153), which are proteins involved in nuclear import of macromolecules, we hypothesized that the variants may therefore affect the nucleocytoplasmic transport function of NUP50. Thus, ongoing work in a neuronal cell culture model is currently being carried out to investigate whether these variants impact 1) the normal localization and arrangement of the NUP50 protein at the nuclear membrane, 2) the efficiency of nucleocytoplasmic transport via the use of reporters, 3) the interaction of NUP50 with known interactants and 4) the localization of proteins (i.e. TDP-43) known to be mislocalized in ALS pathophysiology. Taken together, these first results will shed light on how NUP50 alteration may confer risk to developing ALS, potentially via nucleocytoplasmic transport deficits, which are already considered to be a contributing factor to the disease.

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 *et al.,* Common and rare variants in *NUP50* are risk factors for ALS-FTD, *in review* (2021).

Keywords : NUP50 variants, nucleocytoplasmic transport

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PO 1.5 : LE POISSON-ZÈBRE : UNE AIDE RAPIDE À L'INTERPRÉTATION DES VARIANTS RARES DANS LA SLA

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La Sclérose Latérale Amyotrophique (SLA) est une maladie entraînant le décès en 3 ans par paralysie progressive des quatre membres, de la parole, de la déglutition puis de la respiration. Elle est due à une mort progressive des motoneurones centraux et périphériques. L'étude des familles de patients atteints de SLA permet de distinguer les formes familiales (10%) des formes sporadiques (90%). Quatre gènes majeurs ont été identifiés, impliqués dans environ trois quarts formes familiales et 10 à 20 % des formes sporadiques Cependant, une multitude de gènes sont impliqués dans la maladie ce qui rend difficile l'identification de chaque variant. Les variants rares détectés par les diagnostics moléculaires peuvent être difficiles à interpréter. Des critères de classification ont été proposés par l'ACMG (American College of Medical Genetics and Genomics). Parmi eux, les données fonctionnelles sont considérées comme un élément fort, pouvant permettre de reclasser des variants de signification incertaine (VUS) ou de renforcer la pathogénicité de variants probablement pathogènes. C'est dans cette optique que nous avons mis au point un test fonctionnel d'aide à l'interprétation de

la pathogénicité de ces derniers via l'utilisation de poisson zèbre (Danio rerio). L'approche repose sur une injection d'ARN messager, contenant la mutation à tester, dans la cellule-œuf. La surexpression de variant humain pathogène dans les embryons de poisson provoque des défauts de locomotion à 2 jours ainsi que des anomalies des projections axonales. Ces défauts ne sont pas observés sur les poissons témoins. Les tests ont été validés pour 4 variants du gène SOD1. Nous avons ainsi poursuivi les expériences sur 10 nouveaux variants de SOD1 et 10 variants du gène FUS. Nous proposons ici l'utilisation du poisson-zèbre comme un test original et rapide afin d'aider à l'interprétation des variants rares chez des patients atteints de SLA.

Mots clés : SLA, Génétique, poisson zèbre

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PO 1.6 : IDENTIFYING MOTOR NEURON SPECIFIC ALTERATIONS IN FUS DELETION MUTANT ZEBRAFISH MODEL OF ALS

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Background: 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 report 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 observed in this model. These behavioral deficits were accompanied by anatomical defects, including reduced motor neuron length and fragmentation of neuromuscular junctions (NMJ). The motor deficits were significantly restored by expression of human FUS.

Methods: We have developed techniques to specifically purify neurons by FAC sorting and to perform various omics analysis. By crossing the FUS deletion mutants with the motor neuron specific marker (hb9), we have performed an extensive proteomic analysis of purified motor neurons to validate molecular markers that are deregulated upon FUS deletion.

Results: Importantly, we have identified several dysregulated proteins that are altered in heterozygotes (+/-) and homozygous (-/-) lines. Validation of these altered markers in other pertinent models (iPSCs, mouse model) is underway.

Conclusion: The zebrafish model will provide specific alterations at the motor neuron level and 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, zebrafish, proteomics

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SESSION 2 : BIOMARQUEURS ET THERAPIES DANS LES MALADIES DU NEURONE MOTEUR

- **Conférence invitée**

C2-Conférence : NEUROVITA, UNE NOUVELLE APPROCHE THERAPEUTIQUE POUR LA SLA ?

Monique LAFON(1), Christophe PREHAUD (1), Delphine BOHL (2), Séverine ANDRE (3)

Institut Pasteur, NeuroImmunologie Virale (2) Institut du Cerveau et de le Moelle (3) Neurophoenix

- **Présentations sélectionnées à partir de l'appel à communication**

PO 2.1 : SPINAL CORD MRI FOR TRACKING OF EARLY DEGENERATION IN C9ORF72 ASYMPTOMATIC CARRIERS: A LONGITUDINAL STUDY

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) share genetic susceptibility and a large portion of familial cases are due to *C9orf72* gene mutations. Brain and spinal cord (SC) imaging studies in asymptomatic *C9orf72* carriers have demonstrated white (WM) and grey matter (GM) degeneration up to 20 years before the expected symptom onset[1,2].

The objective of this study is to longitudinally describe, using quantitative MRI, the evolution of spinal cord (SC) degeneration over 36-months observation time in a cohort of asymptomatic *C9orf72* mutation carriers.

Methods: 40 asymptomatic *C9orf72* carriers (C9+) were enrolled in the study. Each subject underwent a 3T cervical SC MRI at baseline, after 18 and 36 months. Quantitative measures of GM and WM atrophy and DTI parameters (FA, AD, MD, RD) in the cortico-spinal tracts (CST) were computed.

We used linear mixed-effects models (LMEMs) to test for significant differences in MRI parameters between time points. We employed the following terms as fixed effects: time point (from T0 up to T3), baseline age, gender, genetic status and interaction terms between time point and age, and between time point and genetic status. Random intercept terms for participants were included in the model.

Results: Mean age at inclusion was 41.18 years +/- 11.46, 18 subjects were male and 22 female. No significant difference in GM and WM cross-sectional area was observed over the three time points ($p > 0.05$). A significant progressive reduction of fractional anisotropy (FA) in the pyramidal tracts was observed over time with a significant difference between the baseline and the 36-months evaluation ($p = 0.04$). No other significant modifications in DTI parameters in the cortico-spinal tract were detected.

Discussion: Cervical SC imaging of *C9orf72* hexanucleotide carriers detect a progressive pyramidal tract FA reduction which seems to be continuous but not linear.

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PO 2.2 : CHARACTERIZATION OF NOVEL CELL-SPECIFIC P2X4 TRANSGENIC SOD1 MICE TO UNRAVEL P2X4 RECEPTOR FUNCTIONS IN ALS AND ITS POTENTIAL USE AS A BIOMARKER

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neurons death. The aggregation of misfolded proteins such as SOD1 or TDP-43 is the pathological hallmark of ALS and has been associated with neuroinflammation and cellular degeneration. P2X4 receptor (P2X4), is a non-selective cationic ATP-gated channel involved in various diseases such as chronic pain and ALS.

In this study, we observed an upregulation of P2X4 in spinal microglia of $SOD1^{G93A}$ ALS mouse model (SOD1) over the disease progression. In addition, we observed a particular surface increase of P2X4 in peripheral macrophages of SOD1 mice before the onset of symptoms, which positions P2X4 as a putative biomarker of ALS. We demonstrated that this aberrant increase in surface P2X4 density is due to specific impairments of P2X4 endocytosis machinery by misfolded mutant SOD1 proteins. To better understand P2X4 functions in ALS, we generate double transgenic SOD1 mice expressing either P2X4 internalization-defective knockin gene (SOD1:P2X4KI) or lacking the P2X4 gene (SOD1:P2X4KO). Surprisingly, both the absence of P2X4 or the expression of non-internalized P2X4KI in all cells naturally expressing P2X4 show the same positive outcome on motor performance and survival of SOD1 mice. This paradoxical output may point out a complex cell-specific and time-dependent roles of P2X4, so far unexplored.

To address the cell-specific function of P2X4 in ALS pathogenesis, we have developed new transgenic SOD1 mice, expressing conditional either knock-in non-internalized P2X4 (cP2X4KI) or knock-out (cP2X4KO) selectively in macrophage/microglia or neurons. We are currently characterizing these novel genetic tools by biochemical, cell culture and behavioral approaches. Furthermore, flow cytometry experiments are conducted in order to detect surface and intracellular human P2X4 in

peripheral blood mononuclear cells from ALS patients. This work may define P2X4 as a useful biomarker for ALS patients and cellular target to fight against this disease.

Key words: P2X, transgenic mice, biomarker

Funding: This project is supported by the ARSLA , FRC, LabEx BRAIN and ANR

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PO 2.3 : CHARACTERISATION OF A THERAPEUTIC APPROACH TO DELIVER INTRABODIES TARGETING INTRACELLULAR TDP-43 IN ALS

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Amyotrophic Lateral Sclerosis (ALS) is an incurable progressive neurodegenerative disease that affects motor neurons. One hallmark of ALS is the presence of toxic 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.

Our lab previously identified single chain variable fragment (scFv) clones exhibiting TDP-43-specific affinity. *In silico* binding prediction revealed the potential binding sites of the scFv's including the N- and C-termini and RRM1/2 of TDP-43. Their interaction with TDP-43 was confirmed using ELISA and Surface Plasmon Resonance (SPR; K_D=3.1E-9). Immunofluorescence and MTT reduction assays demonstrated the non-cytotoxicity and robust expression of the scFv's, respectively. Western blot results suggest that the scFv's decreased the expression and aggregation of TDP-43.

To enhance the efficiency and specificity of the scFv's delivery to target cells, we assembled them into PEGylated superparamagnetic iron oxide nanoparticles (SPIONs). Different mass ratios of SPION to scFv were tested for their size, zeta potential, and scFv retention capacity. The PEG-SPION-scFv formulations with a hydrodynamic diameter smaller than 200 nm and a neutral zeta potential were selected and added to HEK293T cells medium to test their internalization. Flow cytometry and western blot analyses confirmed the cellular internalization of the SPIONs and scFv's, respectively.

Our results confirm the binding of our scFv's to TDP-43 and suggest a potential role in counteracting TDP-43 pathology. To our knowledge, this is the first time that scFv's are complexed to SPIONs for targeted delivery. Further studies will assess the toxicity of PEG-SPION-scFv and their effect on TDP-43 pathology in both *in vitro* and *in vivo* ALS models. We will further evaluate their effect on processes altered by TDP-43 proteinopathy including energy metabolism and RNA metabolism.

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Key words: ALS, TDP-43, scFv.

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PO 2.4 : NUTRITIONAL AND NEUROLOGICAL STATUS OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS WITH AN EARLY INITIATION OF NON-INVASIVE VENTILATION: ASSOCIATED FACTORS AND IMPACT ON EVOLUTION OF THE DISEASE

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Introduction: Non-invasive ventilation (NIV) is a standard care in Amyotrophic Lateral Sclerosis (ALS). It can slow down the deterioration of respiratory function, improve quality of life and survival. The identification of factors associated with an earlier initiation of NIV may allowed to optimize care and quality of life. The aims of the study were to determine the factors associated with early NIV initiation and to compare patient's parameters and survival.

Methods: ALS patients followed in Limoges's reference centre between April 2006 and December 2021 were included. They underwent neurological, nutritional and respiratory evaluations at diagnosis and during follow-up. Three groups were made according to the time of NIV initiation after diagnosis (before 6th month, after the 6th month, no initiation). Statistical analysis was done with the ANOVA, Kruskal-Wallis and Chi² tests then multinomial logistic regression was used for associated factors and the Cox model for survival analysis.

Results: Three hundred and sixty-four patients were included, with a median age of 64 years at diagnosis and a M/F sex ratio of 1.3. Among the 259 patients for whom NIV was initiated, 94 patients (36.3%) had early initiation. In multivariate analysis, one point of initial BMI was positively associated (OR 1.1; p = 0.018) whereas one point of FVC was negatively associated to early initiation (OR: 0.96; p<0.05). In survival analysis, age at diagnosis was associated with risk of death (HR:1.02; p < 0.001). Male gender, fat mass, ALSFRS-R, initial FVC were associated with survival (HR:0.58; p= 0.004, HR:0.97 p = 0.001, HR: 0.96; p = 0.002 and HR: 0.98; p < 0.001 respectively).

Conclusion: In our study, early NIV initiation concerned more than one third of ALS patients. An increase in usual BMI was associated with early NIV initiation. However, in multivariate analysis, early initiation had no impact on patient survival.

Keywords: amyotrophic lateral sclerosis (ALS), Non-invasive Ventilation (NIV), survival

Funding: none

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PO 2.5 : DOES REGULAR CAFFEINE CONSUMPTION IMPACT COGNITION IN AMYOTROPHIC LATERAL SCLEROSIS ?

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Caffeine, the most consumed psychoactive substance worldwide, positively impacts the risk to develop Parkinson's disease and Alzheimer's disease. Compelling epidemiological evidence also support that regular caffeine consumption modulates synaptic plasticity and reduces cognitive decline in ageing, Alzheimer's disease and other neurological/neuropsychiatric conditions.

Amyotrophic Lateral Sclerosis (ALS), a fatal and devastating neurodegenerative disease, is characterized by the loss of corticospinal neurons and motor neurons within the brainstem and spinal cord leading to death within 2-5 years following diagnosis. About 50% of ALS patients also develop non-motor symptoms encompassing cognitive and behavioral changes that appear before or after motor onset. While few studies addressed the impact of habitual caffeine on ALS risk, its impact on the progression of the cognitive disorders has been overlooked.

To address this question we used the Pulse cohort, a prospective multi-centric and multi-modal French cohort following ALS patients from diagnosis up to end of life. Among the 463 patients included, 358 filled out a detail consumption caffeine survey. The link to ALS phenotype, ALSFRS scale, cognitive (ECAS) scores was then investigated.

We observed a significative correlation between regular caffeine consumption and ECAS ($p<0.01$, $r=0.19$) within the 358 patients' population that was more pronounced within the population presenting with bulbar phenotype ($n=78$, $p<0.01$, $r=0.43$). In the later, we also observed a trend for a longer survival linked to the caffeine consumption ($p=0.08$).

We demonstrate for the first time a positive impact of the regular caffeine consumption on cognitive function in ALS patients, predominantly on the bulbar form.

Mots clés : caffeine, ALS, cognition

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PO 2.6 : MOTOR NEURON MORPHOGENESIS IS CONTROLLED BY PHOSPHOINOSITOL SIGNALING TO THE ACTIN CYTOSKELETON

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In motor neuron diseases such as ALS or SMA, motor neuron types with distinct morphological and functional properties differ in their vulnerability to the degenerative process. To study motor neuron morphogenesis, we here isolated motor neurons innervating hindlimb muscles (LMC-MNs) or innervating axial muscles (MMC-MNs) by fluorescent-activated cell sorting (FACS) from embryonic mouse spinal cord [1] and analyzed them under defined *ex vivo* conditions. We demonstate that LMC-MNs develop larger cell bodies and longer axons with less terminal branches than MMC-MNs.

We demonstrate that these differences are intrinsically encoded and associated with the differential expression of genes involved in phosphoinositol synthesis and signaling such as SGMS2, DGKβ, CDS1, PI5P-4K and PLCε. We further show that the transcription factor FoxP1 inhibits Hb9 leading to de-repression of DGKβ (diacylglycerol kinase beta) triggering in turn actin polymerization as well as axon and cell body growth. These data show how transcriptional codes can fine tune the morphogenesis of motor neurons through differential phosphoinositol signaling to the actin cytoskeleton. These data may provide new insights into the differential vulnerability of motor neuron types in degenerative motor neuron diseases.

[1] S Schaller et al. Novel combinatorial screening identifies neurotrophic factors for selective classes of motor neurons. Proc Natl Acad Sci U S A. 2017 Mar 21;114(12):E2486-E93. PMID: 28270618. doi: 10.1073/pnas.1615372114

Mots clés: motor neuron morphogenesis, phosphoinositol signaling, cytoskeleton

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SESSION ARSLA

- **Présentations invitées sur projets financés par l'ARSla**

PO A1 : UNDERSTANDING THE TOXICITY OF ALS-ASSOCIATED KIF5A MUTANT TO MOTONEURONS

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KIF5A gene has recently been linked to amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease caused by the selective death of motoneurons. However, the mechanisms through which ALS-causing KIF5A mutations contribute to ALS pathogenesis remain elusive. Our aim is to characterize and understand the effects of KIF5A mutation on motoneuron functional integrity. To this end, we used *Drosophila melanogaster*, a potent experimental model that offers a wide range of powerful genetic tools to explore spatially and temporally the pathogenic consequence of KIF5A mutant on the motor system. We here show very encouraging preliminary data about the toxic action of KIF5A on motoneurons. Indeed, when expressed in motoneurons at the larval *Drosophila* stage, the KIF5A mutant induces morphological changes at the neuromuscular junction, alteration of larval locomotion as well as altered synaptic transmission. Further investigations will be necessary in order to better understand the pathogenetic mechanisms involved and to outline new therapeutic directions.

Mots clés : KIF5A, Drosophila, axonal transport.

Financement : ARSLA R19101FF

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PO A2 : "OMICS" PROFILING OF PLASMA-DERIVED EXOSOMES FROM ALS PATIENTS: THE SEARCH FOR BIOMARKERS AND TARGETED THERAPY

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Two urgent and unmet needs in ALS are the identification of reliable diagnosis biomarkers and the development of efficient therapy. Interestingly, brain-derived extracellular vesicles (EVs) can be found in plasma and used as a direct read-out of the status of the central nervous system. Studies suggest that EVs have a role in disease progression, since they carry pathological proteins as cargoes. Here, we aimed to identify EVs proteins cargoes from ALS patients to identify putative biomarkers. Plasma EVs were obtained from subjects included in the protocol METABOMU (NCT02670226). For proteomics analysis, EVs (10 controls and 10 ALS patients) were purified trough Size Exclusion Chromatography. Nanoparticle Tracking Analysis (NTA) revealed no differences regarding average size of EVs (Controls: 141.4±9.75nm; ALS: 16.5±13.2nm; mean±SD) nor concentration of particles (Controls: $4.61x10^{10}$ particles/mL; ALS: $4.25x10^{10}$ particles/mL). Interestingly, ALS- and controls-derived EVs presented different Zeta potential (Controls: -22.53±4.3mV; ALS: -8.01±7.9mv;

meand±SD; p=0.001 Unpaired *t* test). Proteomics analysis, performed by LC-MS/MS followed by TimsTOF Pro Mass Spectrometer, revealed 203 proteins significantly different between groups (Padj<0.05). Further analysis with PLGEM algorithm revealed 45 different proteins (p<0.05), 35 being increased in ALS patients in comparison to controls' EVs while other 10 proteins presented higher levels in Controls-EVs. We aimed to confirm these alterations in another cohort of ALS and Controls-derived EVs. EVs (10 controls and 7 ALS patients) were extracted from plasma with the Total Exosome Isolation kit (ThermoScientific) and subjected to immunoblot analysis. Results are currently being analyzed. Metabolomics and lipidomics analysis will be performed to identify the different composition between between Controls- and ALS-derived plasma EVs and confirm the potential of EVs as diagnosis biomarkers. Identification of such differences represent an opportunity for development of targeted therapy based on biomedicaments against EVs carrying pathological proteins. Such targeted therapy has the potential of halting disease progression.

Key-words: proteomics, biomarkers, extracellular vesicles

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PO A3 : FONCTION MOTRICE ET SENSORIELLE DE LA VOIE CORTICOSPINALE CHEZ LE RONGEUR

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La voie corticospinale est particulièrement affectée par la Sclérose Latérale Amyotrophique (SLA), une maladie neurodégénérative mortelle résultant de la dégénérescence conjointe de cette voie et des motoneurones dans la moelle épinière. Il est habituellement admis que la principale fonction de la voie corticospinale (CST) est le relai de la commande motrice. Cependant les connections directes cortico-motoneuronales sont minoritaires (voire absentes, chez le rongeur) par rapport aux connections du CST avec des interneurones spinaux. De plus, il est connu que le CST peut avoir d'autres fonctions, tel que contrôle des entrées sensorielles (« sensory gating », que nous appellerons « contrôle sensoriel »). Le contrôle sensoriel consiste en un filtrage différentiel des entrées sensorielles dès leur entrée dans la moelle épinière, qui permettrait d'augmenter le gain des informations les plus pertinentes pour permettre l'exécution de mouvements fins.

Dans cette étude, nous montrons de façon inattendue que le contrôle sensoriel est la fonction essentielle du CST lombaire chez la souris, alors que la commande motrice est principalement relayée par des voies non CS. En investiguant chaque étage des voies corticofuges pour leur rôle dans la commande motrice et le contrôle sensoriel, nous montrons que ces deux fonctions prennent leur origine dans la même zone du cortex, mais suivent ensuite des voies ségrégées. La commande motrice des pattes arrières est principalement relayée par des centres moteurs supraspinaux sous-corticaux, et n'emprunte que de façon anecdotique la voie CS, et ce via les circuits propriospinaux dans la moelle rostrale. Par contre, les neurones CS sont essentiels pour le contrôle sensoriel et agissent directement via une population d'interneurones lombaires.

Nous présenterons aussi des données préliminaires obtenues chez des souris SOD1 G93A, où nous avons étudié la capacité du CST à relayer la contraction musculaire vs. moduler les entrées sensorielles à un stade présymptomatique (P50).

Mots-clés : corticospinal, sensoriel, moteur

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PO A4 : TDP-43 REGULATION OF ACETYLCHOLINESTERASE SPLICING AND NEUROMUSCULAR JUNCTION STABILITY

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One of the earliest and most prevalent pathogenic features in ALS is the structural and functional dismantling of the neuromuscular junction (NMJ). While recent evidence has implicated widespread dysregulation of RNA processing as a common feature in ALS patients, the relevance of specific RNA targets to the NMJ defects remains to be defined. The RNA and DNA binding protein TDP-43 is a pathological marker and a genetic cause of ALS. Transcriptomic analysis of muscle biopsies from patients has identified a set of key transcripts and splicing factors involved in NMJ stability that are deregulated early in disease, including Acetylcholinesterase (AChE), Collagen Like Tail Subunit of Asymmetric Acetylcholinesterase (ColQ) and the TDP-43 interaction partner, Serine/arginine-Rich Splicing Factor (SRSF2). In this project, we have tested the contribution of TDP-43 to AChE transcript regulation. Using in vitro and in vivo models we have determined that TDP-43 functionally interacts with splicing factors SRSF1 and SRSF2 to control a key switch of splice variants of the AChE transcript that can alter its role at the NMJ, leading to fragmentation and loss of synaptic boutons. In this project we extend and consolidate the understanding of the role of TDP-43 in the NMJ organization and identify AChE as a contributing factor in the early pathological defects of ALS.

PO A5 : EXCITATORY ACTION OF GABA/GLYCINE SYNAPTIC ACTIVITY IS FAVORED IN PRENATAL SOD1^{G93A} MOTONEURONS

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We have previously shown that prenatal spinal motoneurons (MNs) of the SOD1^{G93A} mouse model of amyotrophic lateral sclerosis are hyperexcitable and exhibit an altered chloride homeostasis with a more depolarized chloride equilibrium (E_{Cl}). Here, we aimed to verify whether low frequency depolarizing GABAergic/glycinergic postsynaptic potentials (dGPSPs) exert excitation in SOD1^{G93A} MNs before switching to inhibition at high frequency. Such dual effect was evidenced using simulation from SOD-like MNs (Branchereau et al. eLife 2019). E_{Cl} was set below spike threshold and electrical stimulations of the ventro-lateral funiculus were performed at different frequency in order

to test the ability of dGPSPs to excite or inhibit the firing activity of fetal E17.5 lumbar spinal MNs from the lateral motor column. Dual effect was more often, but not only, detected in SOD1^{G93A} MNs. WT MNs were classified into two clusters (dual effect or pure inhibition) according to Rm, dual responses being specific to high Rm and pure inhibition to low Rm. This was not the case in SOD1^{G93A} MNs that could express dual, pure inhibition but also pure excitation, whatever Rm value. Simulation showed that pure excitation could be obtained in SOD-like MNs by moving away the inhibitory input from the cell body to dendrites. MNs reconstructions highlighted a distinct morphology between the two WT clusters and VIAAT terminals density / size were smaller in SOD1^{G93A} MNs compared to WT. In conclusion, low density and location of GABA/glycine synaptic inputs, in addition to morphological changes, favor excitatory effects of dGPSP trains in E17.5 SOD1^{G93A} MNs. Our data highlight the early alteration in the integration of inhibitory synaptic events in fetal MNs of the SOD1^{G93A} ALS mouse model.

Key words: SOD1^{G93A} ALS mouse model; inhibitory synaptic events; fetal spinal motoneuron

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PO A6 : APPORT DE L’IRM MUSCULAIRE CORPS ENTIER POUR LE DIAGNOSTIC PRECOCE DE LA SCLEROSE LATERALE AMYOTROPHIQUE : ETUDE DE L’INTERET DE LA SEQUENCE DE DIFFUSION

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Objectif : L’utilité de l’IRM musculaire dans le diagnostic de la SLA a fait l’objet de quelques études lors des dix dernières années. Cependant, la quasi-totalité de ces protocoles se basent sur des séquences conventionnelles T1 et T2 et limitent l’exploration quelques groupes musculaires. A ce jour, seules deux études portaient sur l’apport de l’IRM corps entier dans la SLA, dont une seulement incluant une séquence de diffusion. [1] [2] L’objectif principal de cette étude est d’évaluer l’apport de la séquence de diffusion dans un protocole d’IRM corps entier pour le diagnostic précoce de la SLA. Les objectifs secondaires sont d’une part, de décrire la distribution anatomique des anomalies musculaires à l’imagerie ; d’autre part, de comparer la détection des signes de dénervation à l’IRM et à l’ENMG.

Matériels et méthodes : 15 patients remplissant les critères de SLA possible, probable ou certaine d’après les critères d’El-Escorial révisés ou d’Awaji-Shima et 9 témoins ont été évalués par un protocole IRM corps entier avec des séquences T2 Dixon et diffusion (b50 et b800). 101 muscles ont été étudiés à la recherche d’hypersignaux en pondération Water traduisant une dénervation active, d’une involution graisseuse en pondération Fat et d’anomalies de signal en diffusion. L’analyse de l’imagerie a été effectuée en lecture consensuelle par deux radiologues senior et interne puis en seconde lecture par un troisième radiologue senior. Les résultats consistaient en une description de la prévalence et de la distribution anatomique des atteintes musculaires, une comparaison du nombre de muscles atteints chez les cas et les témoins, une étude des performances diagnostiques de la séquence T2 Dixon Water seule et de la combinaison des séquences T2 Dixon Water + diffusion,

une comparaison de l'IRM et de l'ENMG pour la détection des signes de dénervation et une évaluation de la concordance inter-observateur.

[1] Jenkins, T.M. et al. (2018) 'Imaging muscle as a potential biomarker of denervation in motor neuron disease', Journal of Neurology, Neurosurgery & Psychiatry, 89(3), pp. 248–255.

[2] Jenkins, T.M. et al. (2020) 'Longitudinal multi-modal muscle-based biomarker assessment in motor neuron disease', Journal of Neurology

Mots-clés : IRM corps entier, diffusion, T2 Dixon

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SESSION 3 : PHYSIOPATHOLOGIE DES MALADIES DU NEURONE MOTEUR

- Conférence invitée

C3-Conférence : IMPLICATION DES CELLULES MICROGLIALES MACROPHAGES PÉRIPHÉRIQUES DANS LA SLA

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Les cellules microgliales et les macrophages sont des cellules myéloïdes mais ont des origines développementales différentes. Ainsi, dans les conditions physiologiques, chez l'adulte, les cellules microgliales ne sont pas renouvelées par les cellules myéloïdes provenant de la périphérie. Cependant, au cours de maladies neurodégénératives entrainant une neuroinflammation, l'infiltration dans le système nerveux central (SNC) de monocytes/macrophages de la périphérie, reste controversée. Ainsi, nous avons pu montrer, dans des modèles murins de SLA que l'infiltration des monocytes, dans la moelle épinière était faible et dépendait de la progression de la maladie. Cependant, le motoneurone spinal est un neurone particulier puisque son corps cellulaire est dans le SNC et est entouré de cellules microgliales alors que, son axone s'étend à la périphérie et est entouré de macrophages périphériques. Nous avons montré que les cellules microgliales dans la moelle épinière et les macrophages périphérique, dans le nerf, réagissaient de manière très différente à la dégénérescence du même motoneurone. En remplaçant les macrophages périphériques, exprimant la SOD1 muté par des macrophages plus neurotrophiques, surexprimant la SOD1 non mutée, dans les souris SLA, nous avons montré qu'il était possible de diminuer la réactivité des macrophages périphériques mais aussi des cellules microgliales, en agissant uniquement de la périphérie. De plus, remplacer les macrophages périphériques, au début de la maladie a permis de ralentir la progression de la maladie et d'augmenter la survie des souris SLA. Ainsi, il serait possible d'utiliser les macrophages, directement à la périphérie, pour cibler les motoneurones. Nos objectifs actuels sont donc d'analyser les différences existant entre les macrophages périphériques d'individus atteints de SLA par rapport à des populations contrôles pour isoler des voies à cibler dans les macrophages, depuis la périphérie pouvant servir de biomaqueurs et de futures cibles thérapeutiques.

Mots clé : Neuro-inflammation, celles microgliales, macrophages du nerf, monocytes humains

Financements: ARSLA, ALSA, Equipe FRM, Fondation Thierry Latran, ERA-NET NEURON, NRJ-Institut de France, ARMC, S.L.A.F.R., La longue route des malades de la SLA, un pied devant l'autre.

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- Présentations sélectionnées à partir de l'appel à communication

PO 3.1 : TROPHICITY AND TOXICITY OF MACROPHAGES AND MICROGLIA TOWARDS MOTOR NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a rapidly fatal neurodegenerative disorder affecting motoneurons (MNs). Despite the discovery of several genetic causes, many mechanisms involved in MN degeneration in the various ALS forms and the high number of therapeutic trials based on these findings, there is today no curative treatment. One potential reason for failure of the trials is the high heterogeneity of ALS. Since the inflammatory response to MN degeneration is a common mechanism to the different ALS forms, we focused on inflammation with a particular interest in innate immune cells. Indeed, microglia around MN cell bodies and macrophages in peripheral nerves are activated in ALS patients and were recently shown to react differently to MN degeneration driven by mutant SOD1 in a mouse model [1]. The precise mechanisms controlling inflammatory responses in ALS patient's macrophages and microglia remain to be identified. To answer this question, we used human induced pluripotent stem cells (iPSC) to generate MNs, macrophages and microglia, to analyse and compare trophic and toxic roles of innate immune cells towards MN degeneration. Our results show that both ALS macrophages and microglia respond differently to MN degeneration compared to control ones, with specific defects in the endolysosomal pathway and various dynamic inflammatory secretion profiles across time. We also identified specific inflammatory factors which could be interesting targets for future therapies.

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Mots clés: motor neurons, macrophages, human induced pluripotent stem cells.

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PO 3.2 : UNCONVENTIONAL SECRETION OF TDP-43, A PATHOGENIC DETERMINANT OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the progressive degeneration and loss of upper and lower motor neurons leading to the death of patients within 1-3 years after the onsets.

ALS is a proteinopathy associated with pathological inclusions that are mainly composed of ubiquitinated, hyperphosphorylated and cleaved TDP-43. Interestingly, TDP-43 protein displays many similarities with prion proteins especially in its capacity to form aggregates that propagate from cell-to-cell in a seed-dependent and self-templating manner. Different studies showed that pathological TDP-43 (pTDP-43) can be released in association with extracellular vesicles (EVs) or as free aggregates in the extracellular environment. Little is known about the mechanisms involved in the release of free aggregates. Recently, a new unconventional pathway was identified involving the

Ubiquitin-Specific Protease 19 (USP19), an endoplasmic reticulum (ER) resident deubiquitinase involved in the secretion of pathological prion-like proteins α-synuclein and Tau. Here we explored the role of USP19 on the release of pTDP-43 using HEK293T cellular model. While the expression of USP19-WT strongly enhances the release of pTDP-43 in the extracellular medium, conversely, expression of the ER-unattached USP19ΔTM or its deubiquitinase inactive-mutant (C506S) have no effect. As expected, electron microscopy experiments realized on expressing cells revealed a strong accumulation of amorphous cytoplasmic aggregates structures in USP19ΔTM expressing cells whereas these structures were observed in dynamic late endosomes/lysosomes like compartments in USP19-WT cells. Analyses of conditioned mediums from USP19-WT-pTDP-43 expressing cells through sucrose gradient fractionations revealed that most of pTDP-43 are mainly recovered in dense (1.20-1.26 g/cm^3) fractions. Electron microscopy analyses of positive fractions confirmed the presence of heterogeneous spheroid and elongated coiled protein structures thus suggesting that USP19-WT expression favours the release of pTDP-43 free aggregates-like structures. Last, we found that VAMP7, a v-SNARE located on late endosomes/lysosomes, is important for USP19-mediated release of pTDP-43.

Keywords: USP19, TDP-43, unconventional secretion

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PO 3.3 : OREXIN DEPENDENT SLEEP IMPAIRMENT IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease inexorably leading to an early death. Sleep disturbances have been ascribed to respiratory insufficiency, muscle cramps, spasticity, or restless legs syndrome, all leading to increased wakefulness [1]. A recent pathological study in ALS patients observed decreased neurons immunoreactive for orexin, a neuropeptide highly involved in sleep and metabolic regulation. However, sleep changes have been poorly characterized in ALS, and their relationships to motor symptom onset and disease progression or to orexin neurons remain unknown. Here, we used electroencephalography coupled with metabolic cages to characterize sleep and energy metabolism in two murine models of ALS, Superoxide Dismutase 1 G86R (*Sod1*^{G86R}) and Fused in Sarcoma (*Fus*$^{\Delta NLS}$), which both represent 25% of the familial cases of ALS.

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. While we did not observe an altered number of Orexin-positive neurons in the lateral hypothalamus, Suvorexant®, a drug antagonizing both orexin receptors, was able to increase the REM episodes and decrease the wakefulness in both *Sod1*^{G86R} and *Fus*$^{\Delta NLS}$ mice [2]. Interestingly, *Sod1*^{G86R} and *Fus*$^{\Delta NLS}$ mice displayed an increase in body temperature, energy expenditure and locomotor activity, as well as a lower respiratory quotient that were successfully rescued in both mouse models by the drug repurposing strategy.

Thus, our results show that two mouse models of ALS display sleep and metabolic impairments and provide pharmacological evidence for the involvement of orexinergic neurons in these defects.

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Keywords: Metabolism, Sleep, Amyotrophic Lateral Sclerosis
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PO 3.4 : TDP-43 IS ENRICHED AT THE CENTROSOME IN HUMAN CELLS

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TDP-43 is a ubiquitous protein belonging to the RNA Binding Protein (RBP) family. The protein is mainly localized in the nucleus of the cells where it modulates mRNA metabolism through its N-terminal RNA recognition motifs. TDP-43 plays a key role in the pathophysiology of amyotrophic lateral sclerosis (ALS) [1,2], but the precise mechanisms by which the protein contributes to neurodegeneration remain unclear. In recent years, interest has been growing in the presence of RBPs at the centrosome of cells since nucleic acids and ribosomes have been identified within this organelle [3]. Given the structural and functional characteristics of TDP-43, we hypothesized that the centrosome might be a prime location for the protein in cells. We thus investigated the presence of TDP-43 at the centrosome of cultured cells using specific and resolutive techniques such as centrosome purification and super resolution imaging. Immunofluorescence microscopy first allowed us to observe a centrosomal enrichment of TDP-43 in cultured cells, using different primary antibodies and cell lines. This result was further confirmed by Western blot and fluorescence imaging analysis performed on purified centrosomes. Super-resolution microscopy allowed us to specify that TDP-43 was localized at the pericentriolar matrix of the centrosome. The protein was still associated to the centrosome in pathological conditions (cultured skin fibroblasts derived from ALS patients, TDP-43 pathology induced by MG-132 treatment). Finally, we identified more than 20 centrosomal proteins in the literature that have been reported to be TDP-43 interactors and ALS-related proteins, strengthening the link between TDP-43 and the centrosome. In this study we identified a novel localization of TDP-43 in human cells, opening new perspectives in the understanding of physiological and pathological functions of the protein.

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KEY WORDS
Amyotrophic Lateral Sclerosis (ALS), TDP-43, Centrosome
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PO 3.5 : BRAIN REGIONS INVOLVED IN EMOTIONAL AND MOTIVATIONAL PROCESSES AND THEIR LINK WITH APATHY IN ALS: AN MRI STUDY

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INTRODUCTION: ALS is primarily a motor neurodegenerative disorder. However, half of patients with ALS have cognitive and behavioural impairments. Apathy is the most common manifestation associated with behavioural impairment in patients with ALS [1]. Cortical atrophy of regions involved in emotion and motivational processes is a promising indicator of cognitive and behavioural impairments in neurodegenerative diseases [2, 3].
The aim of this study is to investigate whether there is a relationship between the thickness of cortical regions involved in emotions and motivational processes and apathy scale assessed by a neuropsychological assessment tool, the Lille Apathy Rating Scale (LARS).

METHODS: This study is a supplemental analysis using data collected at the Paris Centre, which is part of the PULSE study, an ongoing observational and prospective multicenter cohort (protocol 2013-A00969-36) in ALS patients. A total of 33 patients were evaluated for apathy with LARS and for cognitive impairment with the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). T1-weighted images were acquired with a 3T Siemens scanner and analysed with Freesufer software to determine the cortical thickness (mm) of the regions involved in emotions and motivations, the insula, the cingulate cortex, and the orbitofrontal cortex.

RESULTS: There were significant correlations between cingulate cortical thickness, particularly in the left anterior cingulate cortex ($r=-0.39$, $p<0.01$), right posteroventral cingulate cortex ($r=-0.33$,

p<0.03), and mean left and right insular cortical thickness (r=-0.45, p<0.03) and apathy score, as measured by LARS at the inclusion. In addition, subjects with high subclinical apathy symptoms showed significantly lower (p<0.02) cognitive score assessed by ECAS compared to subjects with low subclinical apathy symptoms.

CONCLUSIONS: Cingulate and insular cortical thickness is a potentially promising measure of apathy in ALS. Further studies with longitudinal design and larger cohort are needed to investigate the predictive ability of cognitive and behavioural impairments with grey matter thickness of these regions.

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KEYWORDS: ALS, Cortical thickness, Apathy score.

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PO 3.6 : CROSS FREQUENCY COUPLING ANALYSIS IN AMYOTROPHIC LATERAL SCLEROSIS IN RESTING STATE EEG

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An early motor cortex cortical hyperexcitability characterizes both sporadic (sALS) and familial (fALS) forms of Amyotrophic Lateral Sclerosis (ALS). This dysfunction precedes lower motor neuron signs, suggesting to be a powerful biomarker promoting ALS early diagnosis. Electroencephalography (EEG) can be an approach to detect and monitor cortical dysfunction through investigation of the interaction between neural oscillations at different frequencies.

We studied the Phase-Amplitude Coupling (PAC), a type of Cross-Frequency Coupling (CFC) analysis, between slow and fast frequency oscillations, known to be highly dependent on

excitation/inhibition (E/I) balance in the brain cortex [1, 3]. Our aim was to investigate whether PAC is altered in ALS patients and if this alteration can represent an ALS cortical dysfunction biomarker.

We used high density EEG recording protocol (74 channels, 4kHz sampling rate), consisting in 5 minutes recordings of eyes closed and eyes open, at rest, on 26 sALS patients and 27 healthy controls. 5 channels around the cranial vertex (Fz, Cz, Pz, C3, C4 according to the 10-20 system) have been analysed using the Tort et al. method [2], which measures PAC estimating the mean Modulation Index (MI).

Our results indicate that MI for Theta-(slow) Gamma (4-8Hz vs. 30-60Hz) PAC was significantly decreased in ALS patients compared to controls whether the eyes were closed or opened, especially at the level of Fz, Cz, Pz and C3 channels. We also found a link between MI and disease progression rate, with MI more depressed in fast than slow progressors, suggesting that the faster the progression rate is, the greater the Theta-Gamma PAC reduction gets. Our results suggest a neural uncoupling likely linked to E/I imbalance at cortical level in ALS, and support the hypothesis that the Theta-Gamma PAC MI extracted from EEG may serve as new quantitative biomarker of cortical dysfunction during disease progression.

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Key words: Amyotrophic Lateral Sclerosis (ALS), Electroencephalography (EEG), Cross Frequency Coupling (CFC)

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LATE BREAKING NEWS

EFFECTS OF SODIUM PHENYLBUTYRATE AND URSODOXICOLTAURINE (AMX0035) ON THE TRANSCRIPTIONAL AND METABOLIC LANDSCAPE OF SPORADIC ALS FIBROBLASTS (VISIO)

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DERNIÈRES NOUVELLES DE L'ACCÈS COMPASSIONNEL TOFERSEN POUR LES PATIENTS SOD1

Philippe Couratier

FilSLAN

CONFÉRENCE « HORS THÈME »

C4-CONFERENCE : PROPAGATION DES PROCESSUS PHYSIOPATHOLOGIQUES LIES A LA SYNUCLEINE DANS LA MALADIE DE PARKINSON

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La vaste majorité des maladies neurodégénératives sont associées à une accumulation de protéines non dégradées et agrégées. On appelle ainsi ces maladies, protéinopathies. Les synucléinopathies en sont une des composantes, en particulier la maladie de Parkinson. Les caractéristiques neuropathologiques de la maladie de Parkinson comprennent la perte progressive de neurones dopaminergiques du mésencéphale et la formation d'agrégats protéiques, constitués notamment de la protéine α -synucléine. Des preuves expérimentales suggèrent que dans des conditions pathologiques, cette protéine, normalement soluble, adopte un repliement anormal et s'agrège, avec une propension à se propager dans tout le système nerveux central. Dans cette illustration, je présenterai la démonstration expérimentale dans des modèles murins et primates d'une telle propagation qui permet désormais de proposer une explication moléculaire à la progression de la maladie chez l'homme.

TABLE RONDE : QUEL AVENIR POUR LES ASO ?

- ✓ **TR1 – ETAT DES LIEUX DU DEVELOPPEMENT DES ASO DANS LA SLA (FOCUS SUR SOD1)**
  Philippe Corcia
  Tours
- ✓ **TR2 – DEVELOPPEMENT DES FUTURES THERAPIES DANS LA SLA (C9, FUS)**
  Hélène TRAN
  Servier
- ✓ **TR3 – AUTRES MALADIES (HUNTINGTON, SCA)**
  Alexandra DURR
  Paris

SESSION POSTERS

P 1 : MOTOR IMAGERY IN AMYOTROPHIC LATERAL SCLEROSIS: AN FMRI STUDY OF POSTURAL CONTROL

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Background and objectives

The functional reorganization of brain networks sustaining gait is poorly characterized in amyotrophic lateral sclerosis (ALS) despite ample evidence of progressive disconnection between brain regions. The main objective of this fMRI study is to assess gait imagery-specific networks in ALS patients using dynamic causal modeling (DCM) complemented by parametric empirical Bayes (PEB) framework.

Methods

Seventeen lower motor neuron predominant (LMNp) ALS patients, fourteen upper motor neuron predominant (UMNp) ALS patients and fourteen healthy controls participated in this study. Each subject performed a dual motor imagery task: normal and precision gait. The Movement Imagery Questionnaire (MIQ-rs) and imagery time (IT) were used to evaluate gait imagery in each participant. In a neurobiological computational model, the circuits involved in imagined gait and postural control were investigated by modelling the relationship between normal/precision gait and connection strengths.

Results

Behavioral results showed significant increase in IT in UMNp patients compared to healthy controls (Pcorrected < 0.05) and LMNp (Pcorrected < 0.05). During precision gait, healthy controls activate the model's circuits involved in the imagined gait and postural control. In UMNp, decreased connectivity (inhibition) from basal ganglia (BG) to supplementary motor area (SMA) and from SMA to posterior parietal cortex (PPC) is observed. Contrary to healthy controls, DCM detects no cerebellar-PPC connectivity in neither UMNp nor LMNp ALS. During precision gait, bilateral connectivity (excitability) between SMA and BG is observed in the LMNp group contrary to UMNp and healthy controls.

Conclusion

Our findings demonstrate the utility of implementing both DCM and PEB to characterize connectivity patterns in specific patient phenotypes. Our approach enables the identification of specific circuits involved in postural deficits, and our findings suggest a putative excitatory–inhibitory imbalance. More broadly, our data demonstrate how clinical manifestations are underpinned by network-specific disconnection phenomena in ALS.

Keywords ALS, motor imagery, dynamic causal modeling

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P 2 : INVOLVEMENT OF OLIGODENDROCYTES IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND FRONTOTEMPORAL DEMENTIA (FTD) LINKED TO FUSED IN SARCOMA PROTEIN (FUS)

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Oligodendroglial pathology is frequently observed in amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD), two neurodegenerative diseases sharing pathological features and genetic causes. Furthermore, oligodendroglial dysfunction has been demonstrated to contribute to *SOD1* ALS. Interestingly, oligodendroglial FUS aggregates are found in ALS and FTD cases associated with pathological aggregates of the FUS protein (FUSopathies), yet their role in disease process remains unknown. Here, we aim to understand the contribution of oligodendrocytes in the onset, propagation and pathophysiology of FUSopathies. To this aim, we used $Fus^{\Delta NLS/+}$ mice, that are conditional knock in mice expressing a mutant form of FUS with C-terminal truncation, similar to what observed in severe cases of ALS. In this mouse model, we observed an increase in myelin sheath thickness at 10 months of age, associated with an increase in myelin protein levels. Interestingly, the motor and behavioral phenotypes of ***$Fus^{\Delta NLS/+}$ mice*** worsened in upon cuprizone injury, suggesting a compensatory function of increased myelination. To determine whether oligodendroglial expression of FUS mutation was necessary, we rescued the *Fus* mutation only in oligodendrocytes using CNP-Cre mice. In these mice, the myelin phenotype appeared earlier, and motor impairments and FTD-like behavioral alterations were partially rescued. Those results suggest that FUS protein may play a role in myelin protein expression (transcription and/or translation), and that the increase in myelin thickness may be a compensatory mechanism. In a complementary strategy, we generated a new mouse model with *Fus* mutation expression restricted to oligodendrocytes. Mouse cohorts are currently being characterized in order to determine whether expression of the mutation in oligodendrocytes is necessary and/or sufficient to drive motor and behavioral phenotypes. These studies will shed light on the contribution of oligodendrocytes to ALS triggered by *FUS* mutations and FTD-FUS.

Key words : ALS ; FUS ; Oligodendrocytes

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P 3 : EVALUATION OF A CLINICALLY-VALIDATED WEB-BASED ANALYSIS MRI PLATFORM TO PROVIDE BIOMARKERS IN ALS

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Introduction

Studies in ALS showed that diffusion tensor imaging (DTI) allows to detect white matter alterations within the cerebral cortico-spinal tract considered as a proxy of upper motor neuron involvement. We aimed to test whether DTI metrics can discriminate patients with ALS from normal controls with a clinically-validated and CE marked web-based MRI analysis platform.

Methods

3T-MRI DTI acquisitions were performed in 24 ALS patients and 22 sex-and age-matched controls. Images were processed by the platform. Outputs were the Mean diffusivity (MD) and Fractional Anisotropy (FA) in regions selected to encompass the corticospinal tract: the posterior limb of internal capsule (PLIC) and cerebral peduncle (CP). The primary objective was to test whether DTI-metrics were different in ALS patients compared to controls. Secondary objectives were to test whether DTI metrics changes correlated with functional severity (ALSFRS-R) and were abnormal in the subgroup of patients without upper motor neuron (UMN) clinical signs.

Results

MD in the PLIC and the CP were increased in ALS patients compared to controls. Decrease of FA did not reach statistical significance. MD in the PLIC was correlated with the ALSFRS-R score. Compared to controls, MD in the CP was increased in the subgroup of patients without clinical UMN signs.

Conclusion

This study shows that a clinically-validated MRI platform can provide DTI metrics proxies of upper motoneuron degeneration in ALS patients even without clinical signs. This tool could be transferred in a clinical setting for diagnosis, decision-making and clinical trials recruitment.

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Mots clés:

Biomarker; Amyotrophic Lateral Sclerosis; Diffusion-Tensor Imaging

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P 4 : GENDER AND SPINAL/BULBAR ONSET INTERACTION ON ALS PROGRESSION – VALIDATION OF A MULTIVARIATE DISEASE COURSE APPROACH

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Background and Objectives

Most of the studies on Amyotrophic Lateral Sclerosis have used cross sectional or survival methods to measure the influence of covariates on disease evolution. If spinal and bulbar onset have been well studied, gender and its interaction with the site of onset has not been fully covered yet. Here, we used disease course modelling to measure the influence of this interaction on ALS progression speed, age at onset and relative evolution of endpoints.

Methods

We selected patients with repeated observations from PRO-ACT database, divided into 4 groups: spinal men, spinal women, bulbar men and bulbar women. We simultaneously investigated the progression of the four sub scores of ALSFRSr, the percent of forced vital capacity (PFVC) and the body mass index (BMI) using a statistical learning approach to model and compare the progression of each group.

Results

5,968 patients met our inclusion criteria and the cohort was composed of 51% spinal men, 11% bulbar men, 27% spinal women and 11% bulbar women. We found that spinal (resp. bulbar) man progression starts 2.04 +/- 0.93 (4.43 +/- 1.64) years earlier than spinal (resp. bulbar) woman progression. If women ALSFRSr fine motor start progressing 4.67 +/- 0.76 months earlier than for men, women ALSFRSr gross motor start progressing 1.6 +/- 0.63 months later than for men. Bulbar women ALSFRSr bulbar (resp. BMI) start progressing 3.77 +/- 2.48 (10.81 +/- 7.06) months earlier than for bulbar men.

Discussion

Using a multivariable disease course model, we have shown a significant effect of gender in both bulbar and spinal onsets. This may lead to further consideration on clinical trial stratifications and this methodology can pave the way to discriminate fast and slow progressor.

Key words

Conflict of interest

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P 5 : COMBINED TENDON REFLEX AND MOTOR EVOKED POTENTIALS RECORDINGS IN AMYOTROPHIC LATERAL SCLEROSIS

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This retrospective (case-control) collaborative, bicentric, study aimed to determine the diagnostic utility of magnetic transcranial stimulation motor evoked potentials combined with patellar tendon reflex recording in upper motor neuron function evaluation on lower limbs in amyotrophic lateral sclerosis.

In ninety-seven patients, all deceased from amyotrophic lateral sclerosis, motor evoked potentials and T-responses were recorded from their quadriceps muscles in the course of their illness. Patients were distinguished according to their patellar reflex briskness at their first clinical examination. Electrophysiological parameters were compared with those of 100 control subjects from a previous study to derive sensitivity and specificity of each of these parameters, alone or in combination. Correlations studies of these parameters were also performed, either with each other, or with patients' clinical characteristics.

The central motor conduction time yields the highest sensitivity (82%) and specificity (93%), allowing twice more detection of upper motor neuron dysfunction than clinical examination, and being more altered in late stages of the disease. The T/MEP amplitude ratio is slightly less sensitive but better

separates patients according to their patellar reflex briskness and might help distinguish physiological from pathological hyperreflexia, the relationship between T and motor evoked potentials amplitudes being inversed in patients as compared with controls. This parameter is not correlated with disability and survival, contrarily to conduction time parameters. The method better detects asymmetry of UMN dysfunction than clinical examination alone.
Motor evoked potentials combined with patellar T-reflex on lower limbs is a powerful biomarker of upper motor neuron loss in amyotrophic lateral sclerosis, coupling of T/MEP amplitude ratios with MEP latencies allowing twice more ALS diagnoses to be made at time of examination than clinics alone, with a sensitivity of 76% and a specificity of 93%. Further studies are needed to precise criteria of electrophysiological hyperreflexia and to expand this simple and painless technique to exploration of proximal upper limbs and bulbar territories.
Keywords: upper motor neuron signs; transcranial magnetic stimulation; T response

P 6 : INVOLVEMENT OF INHIBITORY NEURONS IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA LINKED TO FUSED IN SARCOMA

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder which primarily affects motor neurons, yet multiple evidence suggest that inhibitory neurons are involved in the disease. Indeed, patients show impaired intracortical inhibition prior to motor symptoms onset, and post-mortem studies highlight molecular alterations of cortical and spinal inhibitory circuits. Our laboratory recently identified inhibitory defects in an ALS-FTD mouse model linked to Fused in Sarcoma (FUS). Mutations in *FUS*, encoding for a ubiquitous and multifunctional DNA/RNA-binding protein, cause severe forms of ALS, particularly when FUS nuclear localisation signal (NLS) is truncated. This truncation leads to the cytoplasmic mislocalisation of the protein, which is also observed in ALS and FTD patients devoid of mutations. In mice, the ubiquitous NLS deletion 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]. Our objective in this study is then to characterise the contribution of inhibitory neurons to these phenotypes. To conduct this project, we developed two new mouse models using a Cre-Lox recombination technology, mice in which the Cre recombinase expression is restricted to vesicular GABA transporter (VGAT)-positive neurons, and *Fus* knock-in mice bearing LoxP sequences. We validated the recombination efficiency and then generated mice displaying *Fus* truncation solely in inhibitory neurons. Interestingly, when both copies of *Fus* were mutated, we observed a progressive alteration of the post-natal body development, illustrated by reduced weight and limb strength on post-natal day 15. Furthermore, half of the homozygous pups did not survive weaning. In a complementary strategy, we truncated *Fus* in every cell type except inhibitory neurons. For both models, we are currently characterising adult heterozygous mice for motor and behavioural outcomes. Altogether, our study will determine if *Fus* mutation in inhibitory neurons is sufficient and/or necessary to induce ALS and FTD-like symptoms.
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Keywords: Fused in Sarcoma, Inhibitory neurons, ALS-FTD mouse models.
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P 7 : CHARACTERIZATION OF MUTATIONS INTERFERING WITH TDP-43 SELF-ASSEMBLIES

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Transactive response DNA-binding protein (TDP-43) play a critical role in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients while being mislocalized from the nucleus and aggregates within the cytoplasm of affected neurons. TDP-43 is an RNA-binding protein (RBP) expressed mainly in the nucleus with key functions in RNA processing, e.g., regulation of alternative splicing and polyadenylation, mRNA stability, mRNA processing and localization [1]. TDP-43 is thought to aggregate through aberrant liquid-liquid phase separation (LLPS) via the transition of liquid-like RBP condensates into a solid-like state [2]. Aberrant phase transitions may occur in membrane-less organelles (MLOs) such as stress granules where aggregation-prone RBPs are highly concentrated. Several mutations associated with ALS disrupt TDP-43 LLPS pathway and closely correlate with neurodegeneration. To decipher the role of ALS-associated TDP-43 mutations in aggregation, we used an innovative technology developed by our laboratory, the microtubule bench (MTbench) associated with high-content screen (HCS) to reconstitute liquid phases on the microtubules used as a platform in a cellular context [3]. We were able to develop a model to screen the effect of TDP-43 mutations on its ability to self-associate in liquid compartments. This approach allows us to identify critical residues for LLPS formation. Next, the effect of several mutations on distinct TDP-43 physiological functions were investigated. Surprisingly, the mutated residues impacted the subcellular localization of TDP-43, the recruitment of TDP-43 in stress granules and the exon skipping function. The development of small-molecules interfering with TDP-43 LLPS will open new perspectives to understand the link between the formation of TDP-43 liquid compartments and its cytoplasmic pathological aggregation. Finally, it may pave the way for the discovery of new ALS therapeutic agents by correcting the effects of pathological mutants.

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P 8 : MATRIX METALLOPROTEINASE 9: CANDIDATE FOR EXTRACELLULAR SUPEROXIDE DISMUTASE 1 CLEAVAGE IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of upper and lower motor neurons leading to paralysis and death. The most frequent forms are sporadic (90%). The various mutations of the superoxide dismutase type 1 (SOD1) found in familial ALS (fALS) cases induce misfolding and promote its aggregation leading to toxic gain of function. Numerous studies in genetic mice model of fALS have characterized several non-autonomous and autonomous deleterious mechanisms in different cell types including both neurons and glia. In this context, the propagation of misfolded hSOD1 appears to contribute to the dissemination of neuronal injuries. Although the characterization of the structural and biochemical entities of SOD1 responsible for toxicity and propagation is being established, it remains not completely understood. Our preliminary results show that extracellular unconventionally secreted hSOD1 is a substrate for the metalloproteinase 9 (MMP9) in cell culture. Thus, we are characterizing the protein forms of SOD1 generated by MMP9 *in vitro* and evaluate their toxicity. We have found that only mislfolded hSOD1 appears to be cleaved by MMP9 *in vitro*. Interestingly, differential sensitivity of SOD1 mutants to proteolysis seems to be link to disease severity. Finally, we also found that primary mouse motoneuron expressing ALS SOD1 mutant generate extracellular cleaved form of hSOD1. These suggest that some potentially toxic SOD1 isoforms can be generate by extracellular proteolysis in proximity of the vulnerable MMP9+ motoneurons in spinal cord. These results allow us to define the bases for targeted intervention in these ALS transgenic mice and they will provide new knowledge on the proteinopathy aspect in ALS, necessary for the development of new therapies.
Keywords: Amyotrophic lateral sclerosis, SOD1, MMP9.

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P 9 : INTEREST OF ARTERIAL BLOOD GAS PARAMETERS AS PROGNOSTIC MARKERS IN AMYOTROPHIC LATERAL SCLEROSIS

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Although Forced vital capacity (FVC) is recognized as a prognostic factor of Amyotrophic lateral sclerosis (ALS), its determination remains difficult for some patients due to the rapid progression of the disease. As recently described by an Italian group, Arterial blood gas (ABG) parameters (HCO3-, base excess (BE)) could represent a valuable alternative in these patients [1,2]. Therefore, the aim of this study was to evaluate the correlation between ABG parameters and FVC, and the prognostic ability of ABG parameters in a large cohort of ALS patients. This retrospective study included 307 ALS patients from the university hospital of Tours (2008-2022) with FCV and ABG parameters available at the time of diagnostic. All demographical and standard clinical data were collected. Spinal (n=196) and bulbar onsets (n=111) were analyzed separately. First, correlations between ABG parameters and FVC were evaluated. Then, univariate Cox-regression was carried out to determine unadjusted association of each parameter (ABG and clinical data) with the survival. Finally Cox multivariate analysis was performed to evaluate their independent role on survival. HCO_3^-, pO_2, pCO_2, BE, oxygen saturation and oxyhemoglobin were significantly correlated with FVC for both patients with spinal or bulbar onset. Univariate Cox regression showed that HCO_3^-, methemoglobin and BE were associated with the survival in spinal form while only pH was in bulbar form. However, pH in bulbar form was the only parameter that remained significant after adjustment in Cox multivariate regression. These results suggest a correlation between ABG parameters and FVC at the time of diagnosis, and an interdependence of these parameters on survival. The association of pH at diagnosis with survival needs validation on a prospective cohort. These findings support the interest of a longitudinal evaluation of this correlation to finally use ABG parameters to predict FVC in patients for whom it is no more measurable.

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Mots clés: forced vital capacity, arterial blood gas, survival analysis

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P 10 : UTILISATION DE MÉTAHEURISTIQUES POUR DE L'APPRENTISSAGE AUTOMATIQUE DANS LE CADRE DE LA SLA

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Nous travaillons depuis quelques temps sur des algorithmes d'apprentissage pour aider au pronostic de survie de patients atteints de SLA [1]. Ces méthodes d'apprentissage sont confrontées à divers problèmes, notamment celui de la dimension des données (le nombre de caractéristiques) versus le nombre de données disponibles et utilisables. Le risque est alors un sur-apprentissage conduisant à des modèles de recommandations qui généralisent mal sur une population réelle.

Une des solutions adoptées consiste à réduire la dimensionnalité des données. La réduction de dimension se caractérise par la projection de données décrites en N dimensions vers un espace réduit de dimension K < N. L'objectif principal est de préserver le profil initial des données en proposant une représentation plus pertinente et plus compacte.
Plusieurs approches existent pour réduire la dimension : la sélection de caractéristiques qui consiste à ne garder qu'un sous-ensemble des caractéristiques initiales ou l'extraction de caractéristiques qui repose sur une transformation globale des données grâce à une application qui induit un changement de coordonnées.
Confrontés à ce problème de haute dimensionnalité dans des travaux antérieurs sur la SLA nous avons développé une méthode robuste [2] basée sur une sélection manuelle des caractéristiques des patients suivie d'une phase de réduction de dimension par la méthode UMAP.
Nous proposons ici une amélioration significative de cette approche en incluant une phase préalable de réduction automatique de dimension basée sur des méta heuristiques issues du domaine de la recherche opérationnelle. Cette approche, associée à l'apprentissage automatique permet d'obtenir un sous ensemble presqu'optimal de caractéristiques de données initiales de cohortes [3] de patients atteints de la SLA permettant de construire un modèle mathématique de prédiction simple et performant. Notre méthodologie pourrait ainsi contribuer au pronostic individuel, à la planification préalable des soins et à la stratification dans les essais cliniques.

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Mots clés : SLA, Machine Learning, stratification, pronostic individuel

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P 11 : CLINICAL UTILITY OF STRUCTURAL AND DIFFUSION BRAINSTEM ANALYSIS IN PREDICTING BULBAR AND RESPIRATORY DYSFUNCTION

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Background:
Multimodal neuroimaging provides promising sensitive diagnostic and prognostic marker in ALS (1, 2). However, very few neuroimaging studies have focused on examining changes in brainstem regions in ALS. Brainstem imaging provides a unique opportunity to assess respiratory rhythm generators and bulbar lower motor neurons (3). The primary objective of this study is to investigate longitudinal structural and diffusion changes in the brainstem regions patients and their relationship to bulbar and respiratory functions assessed with standardized tools.

Methods:
This study is an ancillary analysis using data from the Paris Centre, part of the PULSE study, an ongoing large French multicenter observational study and prospective multicenter cohort (protocol 2013-A00969-36) in ALS patients. A total of 45 ALS patients, 12 healthy controls, and 4 diseased controls were recruited. T1-weighted and diffusion tensor images were acquired using a Siemens 3 Tesla MRI scanner. Using freesufer software, a Bayesian algorithm was applied to T1-weighted images to segment and estimate midbrain, pons, and medulla oblongata volumes. Diffusion tensor image analysis was performed in collaboration with the BrainTale platform, which includes the BrainQuant module that enables processing of MRI diffusion images of the brain. Clinical variables including demographic data, ALSFRS-R, muscle strength test, respiratory parameters (ALSFRS-R respiratory subscale, Borg scale, spirometry, NIV initiation data, nocturnal oximetry). Data were collected at five time points: baseline, three, six, nine, and 12 months. Neuroimaging, clinical, and demographic analyses were evaluated for group comparison, correlation analysis, and linear regression prediction model analysis.

Discussion
This ongoing study will allow structural and diffusion measurements of the brainstem to be combined with previously studied structural and diffusion measurements of the spinal cord performed by our team on the same cohort to create a predictive model that will more accurately predict decline in respiratory and bulbar function in ALS patients.

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Keywords: ALS, Brainstem imaging, Respiratory dysfunction

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P 12 : DEVELOPMENT OF AMBROXOL FOR AMYOTROPHIC LATERAL SCLEROSIS

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Ambroxol is a generic drug, with good safety, sold in 77 countries as a mucolytic, and has been shown to be an inhibitor of the non-lysosomal glucocerebrosidase GBA2, while being a chaperone of the lysosomal enzyme GBA1, increasing autophagy. Metabolomic studies had shown that GBA2 was specifically increased in spinal cord of $SOD1^{G86R}$ mice [1], with appropriate changes in ceramide and glucosylceramide and depletion of the downstream neurotrophic glycosphingolipid, GM1, associated with denervation. Glucosylceramidase synthase inhibitors are deleterious in this model, while GBA2 inhibitors are beneficial [2]. Ambroxol reversed these changes and also delayed onset of symptoms and prolonged survival. Ambroxol was also effective in $CHMP2B^{intron5}$ mice and $TDP43^{Q331K}$ mice, being the first compound to be active in the three models, protecting GM1 from degradation, having beneficial effects on denervation. The protective effects of ambroxol against glutamate toxicity in purified motoneurones from $SOD1^{G93A}$ mice parallel those of GM1,and are metabolically linked requiring PPARγ activation and changing PGC1α and TDP-43 distribution. The dosing schedule in the animal studies (at 3 mM in drinking water, 3-fold accumulation spinal cord/plasma) are compatible with the pharmacokinetics of ambroxol and are appropriate for clinical use. A six month phase II clinical trial will follow the dose-progression pioneered in Parkinson's disease [3], and is organized by Prs Vucic and Kiernan, supported by Fight MND. The trial will be performed in Australia using ALSFRS-R, time to event, MUNIX, split-hand index, and other end-points, while also following glycosphingolipids using metabolomics. If positive, the trial will allow a re-evaluation of the importance of glycosphingolipid dysregulation in ALS, opening new therapeutic perspectives.

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Mots clefs : ambroxol, glucosylceramidase, metabolomics. michael@speddingresearchsolutions.fr,

P 13 : IMPACT OF PRENATAL METABOLIC VARIATIONS ON CORTICOSPINAL NEURON PRODUCTION, CIRCUIT FORMATION AND PATHOLOGY

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The developmental origin of Huntington's disease has recently been convincingly demonstrated and is proposed for other neurodegenerative diseases [1]. My Ph.D. project aims to start investigating whether ALS might as well arise from a neurodevelopmental impairment, focusing on the relevant population of corticospinal neurons (CSN). We formerly demonstrated that developmental absence of CSN delays disease onset and extends survival in a mouse model of ALS [2]. Preliminary data from the team of our collaborator, Alice Davy, indicate that CSN production is altered upon methionine restriction, as well as in *Dhfr+/-* mice. Methionine and DHFR are important components of the one-carbon (1C) metabolism, which combines the folate and methionine cycles respectively involved in purine synthesis and methylation reactions, and rely on four key enzymes: DHFR, MTHFR, AHCY and MAT2A. Importantly, alterations of 1C metabolism are not only linked to neurodevelopmental impairments but also found in ALS patients and animal models of the disease [3]. Our preliminary data indicate that *Ahcy* expression is upregulated, while *Mthfr* expression is downregulated in CSN purified from the $Sod1^{G86R}$ mouse model of ALS, and identified a putative polymorphism of *AHCY* in ALS. Therefore, we hypothesize that alterations in 1C metabolism could impact the development of the corticospinal tract and contribute to ALS onset later in life. To test this hypothesis, 1C metabolism will be characterized in the developing CSN of mouse models of ALS ($Sod1^{G86R}$ & $Fus^{\Delta NLS}$) mice by the means of epigenetics, transcriptomics and metabolomics. In parallel, the consequences of the genetic ($Dhfr^{+/-}$ mice) or pharmacological (AHCY inhibition) perturbation of 1C metabolism on CSN development will be assessed. In turn, the project may not only inform on the consequences of ALS-related mutations on brain development, but also on developmental environmental/dietary restrictions on the motor system and potentially the onset of sporadic ALS.

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Key words: development, corticospinal neurons, 1C metabolism

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P 14 : ALTERATIONS DES INTEGRATIONS SENSORI-MOTRICES AU STADE PRECOCE DE LA SCLEROSE LATERALE AMYOTROPHIQUE

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Les fuseaux neuromusculaires (FNM) sont des récepteurs de la proprioception musculaire qui envoient des informations sensori-motrices au cerveau lors d'étirements musculaires ou de vibrations focales musculo-tendineuses [1]. Sur le modèle murin SOD1 de la sclérose latérale amyotrophique (SLA), au stade précoce de développement, les fibres sensitives (Ia et II) du FNM sont désorganisées, leur dégénérescence commence au même moment que celle des axones moteurs mais présente une progression plus lente [2]. Chez les patients atteint de SLA, des preuves d'altération sensorielle infraclinique sont apportées par la corrélation entre données électrophysiologiques et données d'IRM microstructurelle [3]. Alors même que l'examen clinique n'évalue pas l'altération des fibres sensitives du FNM, nous pensons pourtant que le FNM est une modalité sensitive altérée dans la phase précoce de la SLA. Pour évaluer les réponses sensori-motrices corticales et sous-corticales liées aux stimulations du FNM chez les patients au stade précoce de la SLA, nous avons conçu un protocole d'IRM fonctionnelle (IRMf) par blocs (20 sec) de stimulations vibratoires (65 Hz) focales musculo-tendineuses sur la main la plus forte (interosseux dorsal et extenseur des doigts), entrecoupés de périodes de repos (15 sec). Nous avons inclus 21 patients (sans altérations sensorielles cliniques et au début de la dégénérescence musculaire de la main stimulée) et 23 sujets sains. Les deux groupes étaient appariés en âge, sexe et latéralité. Les analyses préliminaires des cartes d'activation cérébrale ont révélé une activité perturbée chez les patients par rapport aux témoins. L'activation était en effet significativement augmentée du côté ipsilatéral à la vibration dans l'aire motrice supplémentaire et significativement plus étendue dans le cervelet. Cela suggère que les intégrations sensori-motrices sont altérées dans la phase précoce de la SLA au niveau cortical et sous-cortical. Ainsi, lors d'une tâche proprioceptive, l'augmentation de l'activité cérébrale rejoint le concept d'hyperexcitabilité corticale connu dans la SLA.

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Mots clés :
- Intégrations sensori-motrices
- IRM fonctionnelle
- Fuseau neuromusculaire

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P 15 : STUDY OF SPG11'S SPLICING, IN NEURONAL AND NON-NEURONAL CELLS, BY LONG-READ SEQUENCING

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Motor neuron diseases are heterogeneous neurodegenerative diseases in which can be found hereditary spastic paraplegias (HSP), Charcot-Marie Tooth (CMT) disease and amyotrophic lateral sclerosis (ALS). The SPG11 gene is one of the most frequently mutated genes in the autosomal recessive forms of HSP and also accounts for ALS and CMT cases [1].
The SPG11 gene encodes a ubiquitously expressed 7.8kb transcript representing the full-length and major transcript [2]. This transcript codes for Spatacsin, a 2 443 amino acids protein. Little is known about the regulation of the expression of this gene, however, but some unpublished data from the lab suggest other existing isoforms. The purpose of my PhD project is to identify all isoforms of SPG11 and establish their full expression profiles, first in non-pathological models but also in pathological models of the disease to determine if the presence of remaining isoforms could explain the variability in clinical presentation.
To do that, we developed a long-read total RNA sequencing protocol on the MinION platform of Oxford Nanopore Technologies (ONT). Currently, 3 human samples from 3 different control individuals and 2 different cell types (fibroblasts and motor neurons (MN) derived from induced pluripotent stem cells (iPSC)) were sequenced and primarily analyzed. RNA extracts from fibroblasts, iPSC-derived MN and PBMC of other control individuals, and from SPG11 patients are under processing. Preliminary analysis of the reads mapped to the region of interest using a “by hands and eye” approach allowed us to identify 5 potential isoforms of SPG11 in addition to the known major transcript. These potential isoforms are currently under validation, first at an in-silico level, before analyzing their expression in vitro. Long-term, our objective is to get a better understanding of the dynamics of SPG11’s expression to help unravel the pathological mechanisms of the disease heterogeneity at the clinical level.
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Keywords: Motor neuron disease; transcriptomic; isoforms
Conflicts of Interest: Stevanin G and Leblanc M received support from Biogen
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P 16 : UNE APPROCHE PHILOSOPHIQUE DE LA SLA : REPRÉSENTATIONS ET MODÈLES DE RECHERCHE

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L’identification de la SLA par Jean-Martin Charcot en 1873 apparaît comme un moment fondateur dans l’histoire de la neuropathologie et de la neurologie. Par la méthode anatomo-clinique, Charcot a pu fournir une première description des principaux mécanismes de la maladie en associant des observations cliniques et neuropathologiques. L’histoire de la connaissance de la SLA est souvent réduite à l’histoire de Charcot, dont le travail est commémoré, notamment par l’usage de l’éponyme

« la maladie de Charcot ». Réduire l'histoire à une approche hagiographique peut engendrer une vision erronée du fonctionnement de la recherche sur la SLA et de son évolution au cours du temps. Une approche combinée philosophique, historique et observationnelle de terrain, nous permet d'appréhender l'évolution des concepts et des modèles élaborés. La comparaison des représentations questionne l'évolution des pratiques et des modèles et de leur interprétation, de l'étude de cas princeps aux modélisations, jusqu'à l'usage des modèles animaux.
Mots clés : philosophie - histoire de la médecine - Charcot
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P 17 : A PILOT PHASE II STUDY TO EVALUATE THE EFFECT OF SALBUTAMOL ON WALKING CAPACITY IN AMBULATORY ALS PATIENTS (WALKALS)

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Repositioning of approved drugs allows accelerating the process of drug development in ALS. Salbutamol has a strong rationale in ALS, especially through its effect on neuromuscular junction (NMJ) destabilization, which is an early and central process in motoneuron degeneration, together with acting on other mechanisms (inhibiting protein degradation with an anabolic effect on muscle, stimulating protein synthesis, inducing neurotrophic factor synthesis and release, positively modulating microglial and systemic immune function and improving energy metabolism).
We will implement a 6-month monocenter randomized controlled phase II pilot study comparing oral salbutamol to placebo. The primary objective will be to test the efficacy of salbutamol on walking capacity. Thirty-six ambulant ALS patients will be included in two arms: 1) 18 patients treated with salbutamol (2mg TID during the first 3 months and 4mg TID during the next 3 months); 2) 18 patients treated with placebo. The primary endpoints will be the change in 6 minutes walking test distance (6MWT) at 3 months (2 mg TID) and at 6 months (4 mg TID). Secondary endpoints will be tolerability and safety as well as clinical outcome measures: quantitative muscle testing, revised ALS Functional Rating Scale-r (ALSFRS-r), twelve items MS Walking Scale (12-MSWS), fatigue scale and respiratory function. Surrogate biomarkers will include measure of the decrement to repetitive nerve stimulation, motor unit count (MUNIX), muscle MRI (DIXON sequence), muscle volume assessment by bioelectrical impedance analysis and plasma neurofilament.
The novelties of this therapeutic trial are to target the NMJ abnormalities that are an early pathogenic mechanism in ALS and to use the 6MWT as a primary endpoint. Using a panel of up-to-date and sensitive biomarkers will give the opportunity to detect a signal of efficacy paving the way for a phase 3 trial. Inclusion of patients is planned to start on Q4 2022.
Mots clés: Neuromuscular junction, ALS, salbutamol

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P 18 : INTRACELLULAR PHASE SEPARATIONS TO UNRAVEL THE CAUSAL ROLE OF ALS PATHOLOGICAL AGGREGATES IN HUMAN MOTOR NEURONS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by the progressive degeneration of motor neurons (MNs) leading to fatal muscle paralysis and death within 1-5 years when the respiratory muscles are affected. Despite the large heterogeneity of the disease, 97% of ALS patients exhibit mislocalization and aggregation of the TDP43 protein in the cytoplasm of MNs. 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. 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.

A promising hypothesis suggests that aggregation-prone proteins pass through a condensed liquid intermediate form before solidifying into toxic aggregates. Although very useful, the Liquid-Liquid Phase separation (LLPS) model of neurogenerative disease-related proteins has been studied mainly through *in vitro* experiments. In our lab, we recently created a methodology to induce the formation of LLPS-driven protein condensates with tuneable biophysical and biochemical properties *in cellulo [1,2]*. Here we will present preliminary data showing the adaptation of this strategy to trigger the assembly/disassembly of TDP43 *in cellulo*. In particular, we will show first observations of artificial TDP43 condensates that recapitulate the hallmarks of TDP43 proteinopathy in HeLa cells and iPSC-derived MNs. This approach could offer a relevant tool to investigate the possible causal link between TDP43 inclusion formation and cellular toxicity.

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Key words: TDP43 inclusions, liquid-liquid phase separation (LLPS), proteinopathy.
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P 19 : *MAPT* MUTATIONS CAN CAUSE VARIOUS FORMS OF AMYOTROPHIC LATERAL SCLEROSIS

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Mutations in *MAPT* encoding TAU protein are widely reported in frontotemporal dementia (FTD) associated with tauopathy and rarely linked to Amyotrophic Lateral Sclerosis (ALS). Here we report 2 mutations in the repeat domains of *MAPT,* required for microtubule binding and assembly, identified by whole exome sequencing analysis in ALS patients. The first mutation (P364S), affecting the fourth microtubule binding repeat of Tau, was identified in three families with autosomal dominant transmission of ALS. Segregation of this mutation could be confirmed in two affected cousins. Patients displayed moderate upper motor neuron deficit at 45-65 year-old and severe dyspnea. Two of them presented initial cognitive impairment including hippocampal memory loss. This P364S mutation was previously reported in a patient with sporadic FTD [1], and several relatives of two Slovene families with cognitive decline and various motor deficits [2-3]. Neuropathological examination performed in these Slovene patients revealed composite neuronal tau inclusions containing 3R/4R isoforms [2-3]. In our study, the analysis of P364S patient fibroblasts and muscular biopsy showed no phosphorylated TAU deposits, although some p62 and TDP-43 positive inclusions were evidenced in muscle fibers. The I308T mutant is a novel variant we identified in a 30 year-old patient with upper limb onset and a disease duration of 5 years. He required respiratory ventilation 3 years after the disease onset. The I308T mutant affects the hexapeptide motif responsible for the pathogenic aggregation of hyperphosphorylated TAU into paired helical filaments. After transfection of plasmids expressing mCherry tagged-*MAPT* (I308T or P364S) in the NSC34 murine motor neuron cell line, we observed a discontinuous distribution of TAU along the microtubules. Cellular toxicity of these constructs will be further compared in this model. Our results highlight a novel I308T *MAPT* mutation causing early onset ALS and suggest the analysis of *MAPT* in ALS patients presenting respiratory symptoms.

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Key words: Motor neuron disease, FTD, Tau
Abbreviations: ALS: Amyotrophic Lateral Sclerosis; FTD: Frontotemporal Dementia.
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P 20 : PROPAGATION OF PATHOGENIC DETERMINANTS OF AMYOTROPHIC LATERAL SCLEROSIS IN HUMAN IPSC-DERIVED MOTOR NEURON MODELS

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ALS is a disorder leading progressively to the complete paralysis of patients due to degeneration of motor neurons (MNs). As disease onset is often anatomically localized, a hypothesis is that the disease could spread to contiguous regions from cell to cell. As one major pathological hallmark observed in patient's are accumulations of misfolded proteins, such as TDP-43, SOD1 or FUS, into cytoplasmic inclusions, we propose that these accumulations could lead progressively to cell overload and saturation of conventional degradation pathways, and then to the involvement of a newly identified unconventional secretory pathway dependent on the deubiquitinase USP19. The latter was shown as able to remove ubiquitin residues from misfolded proteins, to transfer these proteins to DNAJC5 chaperones, then into late endosomes, and finally release into the extracellular space with a possible uptake by surrounding cells. However, until now, most studies focused on pathogenic α-synuclein and Tau protein secretions and were conducted with plasmid over-expressing cellular models. To ask whether the USP19-dependent secretion of pathogenic determinants occurs in ALS affected cells, we used iPSC of patients carrying mutations in SOD1, TARDBP or FUS, as well as isogenic or non-related controls, and differentiated these iPSC into MNs. Our first results reveal that misfolded SOD1 proteins, stained with C4F6 and B8H10 antibodies, accumulate in MNs carrying different SOD1 mutations. Interestingly, confocal analysis shows co-localization of misfolded proteins with DNAJC5 in mutant MNs. SOD1 secretion occurred within less than 2 hours in MN cultures. Downregulation of USP19 with siRNA will be performed to assess whether this secretion is USP19-dependent. We are also investigating whether pathogenic TDP-43 and FUS could be secreted by MNs through the same mechanism.
Keywords: iPSC, USP19, misfolded proteins.
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P 21 : NEUROVITA, AN INNOVATIVE BIOMOLECULE, PROMOTES SURVIVAL AND NEURITE REGROWTH OF MOTOR NEURONS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (poster annulé)

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Amyotrophic Lateral Sclerosis (ALS) is characterized by the degeneration of motor neurons (MNs) in patients leading rapidly to paralysis of all skeletal muscles. ALS complex etiology with multiple genetic targets and the involvement of environmental factors makes therapeutic approaches for all ALS patients particularly challenging. Nevertheless, a unifying feature of ALS is the progressive MN degeneration. To target this feature, we propose the innovative bio-drug Neurovita (NV), discovered by the laboratory of Dr Lafon, as a new therapeutic option. NV is a peptide isolated from a virus that promotes the survival of neurons it infects. The NV cellular targets and the activated downstream molecular pathways have been identified and well characterized [1, 2, 3]. NV's properties of keeping alive neurons whose survival is compromised and of allowing the regrowth of injured nerves, have been established.

To study the ability of NV to promote survival of ALS MNs, we model the disease with human induced pluripotent stem cells (IPSC) derived from ALS patients mutated in the 4 main ALS genetic causes (C9orf72, SOD1, TARDBP, FUS), from sporadic cases, and from control subjects. iPSC are differentiated into MNs and then transduced with lentiviral vectors expressing NV or ΔNV. To assess neuroprotective and neuroregenerative properties of NV, we set up scratch-insult conditions by enzymatic treatment to remove MN cells from solid substrate, followed by replating of MNs and analysis at different time-points. MN survival, cell imaging and morphometric analysis are analyzed using the automated Cell-Insight station. First results on mutant C9orf72 and SOD1 MNs suggest that NV can protect ALS MNs from death and promotes axon outgrowth. Experiments are now conducted with other ALS iPSC clones to assess whether NV could be envisaged as a therapeutic option for all ALS cases regardless of the nature of the affected gene or triggering factors.

[1] Préhaud C, Wolff N, Terrien E et al. Attenuation of rabies virulence: takeover by the cytoplasmic domain of its envelope protein. Sci Signal. 2010 Jan 19;3(105):ra5. [2] Khan Z, Terrien E, Delhommel F et al. Structure-based optimization of a PDZ-binding motif within a viral peptide stimulates neurite outgrowth. J Biol Chem. 2019 Sep 13;294(37):13755-13768. [3] Patent 2013/068430 (HIGH MAST2-AFFINITY POLYPEPTIDES AND USES THEREOF).

Keywords: biomolecule, iPSC, motor neurons.

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Conflict of interest: ML is co-founder and scientific advisor of Neurophoenix

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P 22 : DECIPHERING THE ROLE OF TBK1 IN MOTOR NEURONS AND MICROGLIAL CELLS AND ITS IMPLICATIONS FOR ALS PATHOGENESIS

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Mutations in the ubiquitously expressed *TANK-Binding Kinase 1* gene (*TBK1*) are linked to Amyotrophic Lateral Sclerosis (ALS) and act by a dominant loss-of-function mechanism. TBK1 is involved in autophagy and innate immunity, suggesting that *TBK1* mutations could lead to ALS by both cell-autonomous (autophagy deregulation in motor neurons, MN) and cell-non-autonomous mechanisms (altered responses in microglia). To decipher the role of TBK1 in these two cell types, we have generated mice with *Tbk1* deletion specifically in MN or microglia. Surprisingly, using transcriptomic analyses, we found that *Tbk1* deletion induces much more changes in microglia than MN. In MN, loss of *Tbk1* nevertheless leads to increased age-related alterations of the neuromuscular junctions and persistent p62 inclusions in an intriguing MN subpopulation, but it is not sufficient to induce loss of MN. To study the role of *Tbk1* in microglia, we first used primary mouse microglial cells and showed that *Tbk1* deletion decreases their reaction to lipopolysaccharide (LPS) but produces a novel unexpected response to the stimulus. In parallel, we studied microglia isolated from young and aged mice and found that *Tbk1* deletion induces an aged-like phenotype in microglia from young mice. This modified microglia could be more neurotrophic or neurotoxic for MN. To study that, we have started to analyze the effect of *Tbk1*-deleted microglia on chronic MN degeneration in an ALS mouse model. We found no significant effect on survival, but *Tbk1*-deleted microglia tend to delay the worsening of the symptoms of these ALS mice. We are still following the cohort of mice and performing histochemical analyses. Altogether, our results suggest that while *Tbk1* deletion in MN seems deleterious, it could be rather neurotrophic in microglia *in vivo.* This project could help better understand the contribution of pathological MN-microglia interactions.
We would like to present our work as a poster only. (290/300)
Mots clés : TBK1, Mouse model, Microglial cells.
Financements : ARSLA, Neuratris, ARMC, S.L.A.F.R., La longue route des malades de la SLA, Un pied devant l'autre.
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P 23 : DÉVELOPPEMENT D'UNE APPROCHE RNAE (ENHANCEMENT) PERMETTANT D'AUGMENTER LA TRADUCTION DE PROTÉINES D'INTÉRÊT DANS LA SCLÉROSE LATÉRALE AMYOTROPHIQUE

Vossels M (1), Marouillat S (1), Hung D (1), Veyrat-Durebex C (1,2), Blasco H (1,2), Corcia P (1,3), Andres CR (1,2), Laummonier F (1), Zaleta-Rivera K (1) and Vourc'h P (1,2).

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La SLA est une maladie neurodégénérative incurable caractérisée par la mort progressive des neurones moteurs. Pour pallier au dysfonctionnement synaptique survenant précocement dans la maladie, nous avons testé une nouvelle approche thérapeutique basée sur des lcnARN synthétiques,

les SINEUPs, permettant d'améliorer l'efficacité de traduction d'ARNm cibles. Pour cela nous avons utilisé des ARN antisens ciblant dans la région 5'UTR les ARNm codant les synapsines (SYN I et II) et la synaptophysine (SYP), des protéines synaptiques participant à la synaptogénèse et au maintien synaptique. Ces ARN antisens ont été clonés dans un plasmide possédant le domaine effecteur SINE et codant la GFP, puis à partir de ces constructions des vecteurs viraux AAV9 exprimant ces SINEUP ont été produits.
Nos études sur l'effet des SINEUPs au sein de la lignée motoneuronale NSC-34 ont montré une augmentation d'expression des protéines SYN I et SYN II A et B ainsi qu'une augmentation de de la protéine SYP. Nous avons également pu mettre en évidence une augmentation significative de la longueur des neurites pour nos différents SINEUP dans les cellules NSC34 différenciées.
Nous avons également produit un modèle *in vitro* de cultures primaires de neurones d'hippocampe présentant une réduction d'expression des protéines synaptiques cibles, comme observé dans la SLA,. En parallèle, nous avons testé des virus AAV9 exprimant les précédents SINEUPs sur des neurones. Ces expériences préliminaires ont montré une diminution d'expression de SYN et SYP dès 24h après traitement avec le Létrozole qui se prolonge à 72h. Nous avons également observé une expression de nos SINEUP dans les cultures de neurones après 10 jours de culture.
Ces résultats sont très encourageants et pourraient participer à ouvrir la voie vers de nouvelles thérapeutiques dans les maladies neurodégénératives, et dans la SLA en particulier.
Mots clefs : SLA, Synapses, ARN non codants
Remerciements : ARSLA, Studium Centre Val de Loire
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P 24 : ALTERATION OF THE NEUROMUSCULAR JUNCTION AND MODIFICATIONS OF MUSCLE METABOLISM IN RESPONSE TO NEURON-RESTRICTED EXPRESSION OF THE CHMP2BINTRON5 MUTANT IN A MOUSE MODEL OF ALS-FTD SYNDROME.

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CHMP2B is a protein that coordinates membrane scission events as a core component of the ESCRT machinery. Mutations in CHMP2B are uncommon cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) two neurodegenerative diseases with clinical, genetic and pathological overlap. Different mutations have now been identified across the ALS-FTD spectrum. Disruption of the neuromuscular junction is an early pathogenic event in ALS. Currently, the links between neuromuscular junction functionality and ALS-associated genes such as CHMP2B remain poorly understood. We have previously shown that CHMP2B transgenic mice expressing the CHMP2B^{intron5} mutant specifically in neurons develop a progressive motor phenotype reminiscent of ALS. In this study, we used complementary approaches (behavior, histology, electroneuromyography, biochemistry) to determine the extent to which neuron-specific expression of CHMP2B^{intron5} could impact the skeletal muscle characteristics. We show that neuronal expression of the CHMP2B^{intron5} mutant is sufficient to trigger progressive gait impairment associated with structural and functional changes in the neuromuscular junction. Indeed, CHMP2B^{intron5} alters the pre-synaptic terminal

organization and the synaptic transmission that ultimately lead to a switch of fast-twitch glycolytic muscle fibers to more oxidative slow-twitch muscle fibers.
Taken together these data indicate that neuronal expression of CHMP2B^{intron5} is sufficient to induce a synaptopathy with molecular and functional changes in the motor unit reminiscent of those found in ALS patients.

Keywords: CHMP2B^{intron5}, motor neuron disease, neuromuscular junction.
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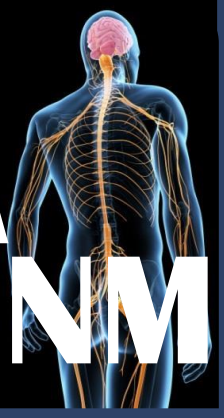

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