10TH ALS AND MND RESEARCH MEETING

October 9 and 10, 2024

ICM, Paris

With the support of

Wednesday 9 October 2024

9:30 - 9:45

Opening: Philippe COURATIER (FILSLAN), Olivier GOY (ARSLA), Claude DESNUELLE (ARSLA)

9:45-11:45

SESSION 1: PATHOPHYSIOLOGY OF MOTOR NEURONE DISEASES
Moderators: Séverine BOILLEE, Véronique PAQUIS

9:45

- Conference: Physiological and multi-omic signatures of cortical impairment in ALS

Caroline ROUAUX
INSERM & Strasbourg University

- 6 oral communications (6 x 15 minutes)
  - **10:15** OC 1.1 - Xhuljana MINGAJ *Imagine Institute, Paris*
  - **10:30** OC 1.2 - Jelena SCEKIC-ZAHIROVIC *German Center for Neurodegenerative Diseases-DZNE, Ulm*
  - **10:45** OC 1.3 - Matthieu RIBON *Paris Brain Institute, Paris*
  - **11:00** OC 1.4 - Killian GHIKH-MIMIETTE *Paris Brain Institute, Paris*
  - **11:15** OC 1.5 - Philippe GOSSET *Institute for Neurosciences, Montpellier*
  - **11:30** OC 1.6 - Caroline RITACCO *Institute for Neurosciences, Montpellier*

11:45-12:00

Break (15 minutes)

12:00-12:30

FILSLAN "Training through research" project Award 2023 (3 x 10 minutes)

- **12:00** Alexia BODIN
- **12:10** Anne FENOY
- **12:20** Jeflie TOURNEZY

12:30-14:00

Lunch buffet / First poster session

14:00-16:00

SESSION 2: BIOMARKERS AND THERAPIES IN MOTOR NEURONE DISEASES
Moderators: Edor KABASHI, Philippe CORCIA

14:00

- Conference: Antisense oligonucleotide therapies for ALS

Philip VAN DAMME
Neurology Department, University Hospital Leuven, KU Leuven, Belgium

- 6 oral communications (6 x 15 minutes)
  - **14:30** OC 2.1 - Laura TZEPLAEFF *Rechts der Isar Hospital of the Technical University Munich*
  - **14:45** OC 2.2 - Hugo MOURIER *MMDN, Montpellier University*
  - **15:00** OC 2.3 - Giorgia QUERIN *Hôpital Pitié-Salpêtrière, Paris*
  - **15:15** OC 2.4 - Mohammed KHAMAYSA *Laboratoire d'Imagerie Biomédicale, Paris*
  - **15:30** OC 2.5 - Yara AL OJAIMI *iBrain Tours*
  - **15:45** OC 2.6 - Marc DIBLING *Paris Brain Institute*

16:00-16:30

Break (30 minutes)

16:30-18:00

ARSLA SESSION (6 x 15 minutes)
Moderators: Pierre-François PRADAT, Cédric RAOUL

- **16:30** OC A1 - Maëlle OLLIVIER *INCIA, Bordeaux*
- **16:45** OC A2 - Luc DUPUIS *CRBS, Strasbourg*
- **17:00** OC A3 - Léa BEDJA--IACONA *iBraiN, Tours*
- **17:15** OC A4 - Chantal SELLIER *CRBS, Strasbourg*
- **17:30** OC A5 - Benoit SCHNEIDER *UMR-S1124, Paris*
- **17:45** OC A6 - Pauline DUC *IGMM, Montpellier*

18:00

Conclusion day 1

From 19:00

ARSLA Awards Evening, ICM (on registration)

Thursday 10 October 2024

9:00-11:00

SESSION 3: MOLECULAR MECHANISMS OF MOTOR NEURONE DISEASES
Moderators: Philippe CODRON, Pascal LEBLANC

9:00

- Conference: Understanding the life cycle of TDP-43: aggregation triggers & toxicity effectors
Magdalini POLYMENIDOU
University of Zurich, Department of Quantitative Biomedicine

- 6 oral communications (6 x 15 minutes)
  - **9:30** OC 3.1 - Coline JOST MOUSSEAU *Paris Brain Institute*
  - **9:45** OC 3.2 - Loan VAILLANT-BEUCHOT *IRCAN, Nice*
  - **10:00** OC 3.3 - Elena PASHO *Institut des maladies génétiques, Paris*
  - **10:15** OC 3.4 - David GENTIEN *AMMICa, Villejuif*
  - **10:30** OC 3.5 - Anaelle BURGARD *Laboratory for Cognitive and Adaptive Neuroscience, Strasbourg*
  - **10:45** OC 3.6 - Jianbo HUANG *INMG-PGNM, Lyon*

11:00-11:15

Break (15 minutes)

11:15-12:15

Conference: Unlocking the Secrets of Minipuberty and GnRH Neurons: From Brain Development to Cognitive Resilience and Beyond
Vincent PREVOT
Development and plasticity of the neuroendocrine brain INSERM University of Lille - CHRU Lille

12:15-13:45

Lunch buffet / Second poster session

13:45-14:00

Announcement of ARSLA awards / Research support actions
ARSLA scientific committee jury :
- Aude-Marie GRAPPERON
- Hélène BLASCO
- David DEVOS
- Pascal BRANCHEREAU

14:00-16:00

ROUND TABLE: What did we learn from recent failures in therapeutic trials in ALS?
Moderators: Claire GUISSART, David DEVOS

14:00

- Recent failures in ALS therapeutic trials, from a clinician's perspective: the challenge of clinical heterogeneity
  Gaelle BRUNETEAU
  Neurology Department, Paris ALS Expert Center, Alliance on Clinical Trials for ALS-MND, Paris Brain Institute (ACT4ALS-MND, ICM), Myology Center For Research, Sorbonne University

14:20

- Learning from failed trials in the past and proposals for the future
  Thomas MEYER
  Charité – Universitätsmedizin Berlin, Center for ALS and other motor neuron diseases

14:40

- Statistical learnings from recent therapeutic trials in ALS
  Ruben VAN EIJK
  University Medical Center Utrecht

15:00

Discussions

16:00-16:30

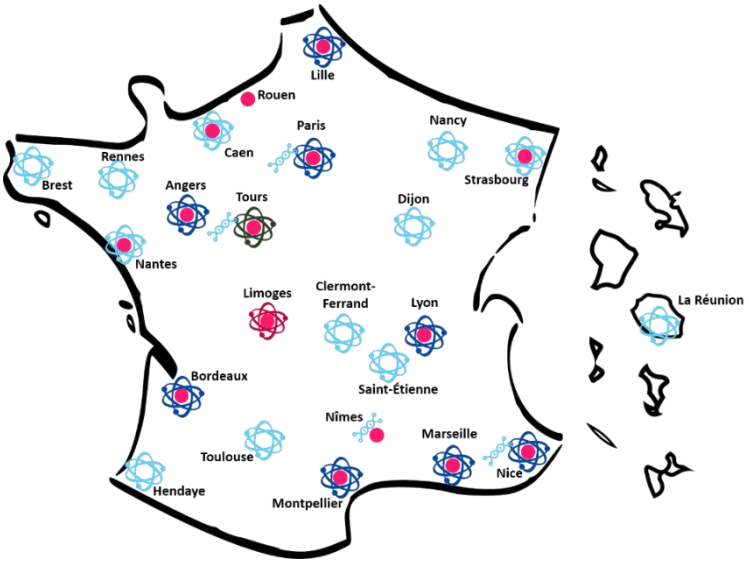
Closing / General conclusion: Philippe COURATIER (FILSLAN)

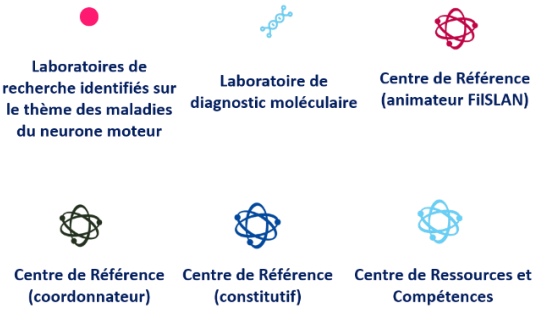
Presentation code: C = conference, OC = oral communication, RT = round table, P = poster

FILSLAN

FILSLAN is the national network for rare diseases: Amyotrophic Lateral Sclerosis and motor neurone diseases. The network was created in 2014 by the Ministry of Social Affairs and Health as part of PNMR 2 and under the responsability of DGOS. Since January 2021, Professor Philippe COURATIER is the national coordinator of the FILSLAN network which is located at the Limoges University Hospital. In 2023, 22 ALS Centers have been labeled.

FILSLAN territorial network





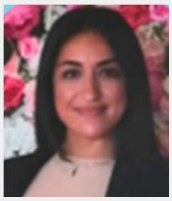
The FILSLAN team

Aurélie THEILLAUMAS BNDMR project, Daniells Andréa ERAZO research manager, Philippe COURATIER national coordinator, Julie CATTEAU project manager and Coline AUPART communication officer

ACT4ALS-MND

National Network for Clinical Research in Amyotrophic Lateral Sclerosis - Motor Neurone Diseases





Gaëlle BRUNETEAU network coordinator, Amandine BORDET project manager, Alizé CHALANÇON project manager

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FILSLAN website

ARSLA

Created in 1985, ARSLA is the French National Association for research on ALS.

It works to discover treatments and a cure for ALS, also to serve and advocate for empower people affected by ALS.

It offers several services to help and support the patients and their families. For example, it provides for free, equipment that improve quality of life - such as communication devices that allows people with ALS to communicate despite all their limitations.

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ARLSA Research

Since ARSLA's creation, it has committed over €14 million to support more than 240 projects. 4 types of projects can be supported by ARSLA.

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- Clinical projects: medical care and global, e-health/information technology, new evaluation criteria, epidemiology/natural history.
- Projects for young researchers: research projects carried out by 4th year students' thesis on ALS.
- Biotech projects: research projects carried by start-ups to develop therapies in ALS.

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EDITORIAL

Since 2015, the FILSLAN rare disease health network has been involved in motoneurone disease research. This year the 10th edition is organized in Paris Brain Institute for 2 days.

The objectives of this meeting is to share and update scientific knowledges on motor neurone diseases. Three different sessions will concern Physiopathology, Biomarkers and therapy and Molecular Mechanisms. Following the call for communications, eighteen oral presentations and 19 posters will be presented, providing a stimulating opportunity for young researchers to share the progress of their work.

Philip Van Damme from Leuven will give a talk on antisens oligonucleotides for ALS. Caroline Rouaux, from Strasbourg, will discuss Physiological and multiomic signatures of cortical impairment in ALS. The topic of understanding the life cycle of TDP-43 will be addressed by Magdalini Polymenidou from Zurich. Vincent Prevot, from Lille will give a transversal view of neuroendocrine brain and will have a talk on unlocking the Secrets of Minipuberty and GnRH Neurons. Finally, we will have an exciting round table on Lessons we learned about recent failures in therapeutic trials in ALS. A discussion will be given by three european experts: Gaelle Bruneteau from Paris, Thomas Meyer from Berlin and Ruben Van Eijk from Utrecht.

Last year's 170 researchers participated. We hope you enjoy this new edition demonstrating the growing interest in ALS and Motoneurone diseases research.

Pr P.Couratier

FROM JR1 to JR10

SUMMARY

Presentation code: C = conference, OC = oral communication, RT = round table, P = poster

SESSION 3: MOLECULAR MECHANISMS OF MOTOR NERONE DISEASES

SESSION 1: PATHOPHYSIOLOGY OF MOTOR NEURONE DISEASES

Moderation: Séverine BOILLEE, Véronique PAQUIS

- **Guest lecture**

C1 Conference: Physiological and multi-omic signatures of cortical impairment in ALS

Caroline ROUAUX

INSERM & Strasbourg university

- **Presentations selected from abstracts submitted**

OC 1.1 - Metabolic modification in cellular and animal models of FUS-ALS

Xhuljana Mingaj (1), Jad Awad (1), Lauaralee Robichon (1), Pierre Cauchy (2), Salim Megat (2), Quentin Raas(1), Maria-Letizia Campanari (1), Anca Marian (1), Ivan Nemazanyy (3), Christine Bole (4) Nicolas Cagnard (5), Isabelle Desguerre (6), Giulia Barcia (7), Luc Dupuis (2), Sorana Ciura (1), Edor Kabashi (1)

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FUS, a major gene mutated in Amyotrophic Lateral Sclerosis (ALS) patients is an DNA/RNA-binding protein, which plays a key role in RNA metabolism. FUS mutations are often associated with juvenile ALS leading to significant muscle weakness and motor neuron degeneration. We previously characterized a stable zebrafish fus mutant transgenic line that replicates the locomotor impairments typical of ALS patients. In this study, we further investigate the impact of FUS deletion on post-synaptic features, revealing disruptions in muscle fiber organization and mitochondrial network integrity. Integrated transcriptomic and metabolomic analyses of both homozygous mutant zebrafish and C2C12 muscle cell lines where FUS expression was depleted showed similar metabolic pathway perturbations. Our results delineate FUS-mediated inhibition of the PPAR-alpha gene by up-regulating the slc2a1 gene, a key glucose transporter facilitating cellular glucose uptake and major deregulation of carnitine metabolism. We identified Acetyl-L-Carnitine as a potent chemical modifier that rescues the motor phenotype and survival rate of FUS -/- zebrafish larvae by counteracting the downregulation of long-chain fatty acid oxidation in mitochondria. Moreover, our results confirm a distinct metabolic shift from oxidative metabolism to glycolysis, reminiscent of the Warburg effect observed in cancer cells and this was evident both in the zebrafish model and C2C12 cells. Indeed, deregulation of the PKM2/PKM1 ratio was observed in these models with TEPP-46, a PKM2 activator, rescuing the motor phenotype, yielding a more homogeneous milder population as compared to the fully paralyzed FUS-

deficient zebrafish. Similar metabolic defects, including carnitine deficits, were also observed in fibroblast cells from a juvenile ALS patient carrying a truncating FUS mutation. Thus, our results indicate that FUS is essential for maintaining muscle structure and proper oxidative metabolism. Importantly, we identify therapeutic strategies that could reverse muscle wasting in FUS driven ALS and could be applicable to related neuromuscular diseases.

Keywords: FUS-ALS, muscle metabolism, lipid oxidation

This project was funded by ARSLA, Association pour la Recherche sur la SLA (Jeune chercheur dotation scientifique 2023).

The authors declare no conflict of interest.

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OC 1.2 - Impairment of hypothalamic MCH neuronal network precedes disease onset and MCH degeneration in ALS mouse model

Jelena Scekic-Zahirovic (1), Stefano Antonucci (2), Chiara Ebner (4), Diana Wiesner (1), Hussein El Hajj (2), Gizem Yartas (1), David Bayer (3) Amela Londo (2), Albert C. Ludolph (1,2), Francesco Roselli (1, 2)*

(1) German Center for Neurodegenerative Diseases-DZNE, Ulm, DE. (2) Department of Neurology, Ulm University, Ulm, DE. (3) CEMMA (Cellular and Molecular Mechanisms in Aging) research training group, Ulm, DE. (4) Molecular and Translational Neuroscience, Master program, Ulm University, Ulm, DE.

Weight loss and hypermetabolism precede the onset of amyotrophic lateral sclerosis (ALS) for years, predict patients' survival and can be mitigated with symptomatic treatment – hypercaloric diet [1]. In ALS patients weight loss was linked to atrophy of hypothalamus [2], and recently to degeneration of hypothalamic melanin-concentrating hormone (MCH) neurons detected *post-mortem* [3]. Yet, mechanisms driving MCH neurons and/or network vulnerability in ALS remain unknown and causal treatment is lacking. To investigate this question, we first showed that symptomatic *SOD1^{G93A}* mice, that are hypermetabolic and rapidly lose weight replicate MCH degeneration, as seen in patients. Among several hypothalamic populations we identified to undergo degeneration in mice, MCH population appeared especially vulnerable with loss of 40%. Next, to investigate whether vulnerability occurs at network level we mapped the brain-wide, monosynaptic inputs to MCH neurons prior and upon their degeneration. To ensure MCH-specific connectivity we used retrograde tracing based on the modified rabies and Cre-inducible helper viruses co-injected into hypothalamus of transgenic *Mch-Cre;SOD1^{G93A}* and *Mch-Cre;WT* mice. Local inputs, connecting hypothalamic nuclei to MCH neurons (primarily inputs from zona incerta-ZI) were lost early in pre-symptomatic mice, prior to MCH loss. In symptomatic mice network loss progressed as detected by reduced inputs from other hypothalamic nuclei and by altered extra-hypothalamic inputs to MCH (from cerebral nuclei). Principal component and hierarchical cluster analysis revealed that impaired connectivity patterns from hypothalamic and cerebral nuclei to MCH were sufficient to separate healthy from diseased animals. Impaired MCH inputs from ZI showed dopaminergic identity, accumulation of misfolded SOD1 and p62 inclusions, and strong surrounded astrogliosis. Thus, MCH network undergoes disease-related disruption in ALS mice, prior to weight deficit and MCH degeneration and is accompanied by disease pathology and inflammation. Ongoing *in vivo* manipulation of MCH neurons/network will define their contribution to ALS and therapeutic potential in preventing neuronal death.

References:
[1] Ludolph A., *et al*. Effect of High-Caloric Nutrition on Survival in Amyotrophic Lateral Sclerosis. Ann Neurol. 2020. doi: 10.1002/ana.25661.
[2] Gorges, Vercruysse P., *et al*. Hypothalamic atrophy is related to body mass index and age at onset in amyotrophic lateral sclerosis. JNNP. 2017. doi: 10.1136/jnnp-2017-315795.
[3] Bolborea M., *et al*. Loss of hypothalamic MCH decreases food intake in amyotrophic lateral sclerosis. Acta Neuropath. 2023. doi: 10.1007/s00401-023-02569-x.
Key words: weight loss, MCH, dopamin
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OC 1.3 - Characterization of blood-derived macrophages of Amyotrophic Lateral Sclerosis (ALS) for therapeutic and diagnostic approach

Matthieu Ribon (1), Adèle Hesters(1)(2), Maria Del Mar Amador(2), Lorraine Bavelier (1), Aude Chiot (1), Gaëlle Bruneteau (2), Timothée Lenglet (2), Philippe Couratier (3) Stéphanie Millecamps (1), Delphine Bohl (1), Christian S Lobsiger. (1), François Salachas. (1)(2), Séverine Boillée (1)

(1) Sorbonne Université, Institut du Cerveau – Paris Brain Institute – ICM - Inserm, CNRS, AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France. S.Boillée Team « ALS causes and mechanisms of motor neuron degeneration » (2) Département de Neurologie, Centre de référence SLA Ile de France, Assistance Publique Hôpitaux de Paris (APHP), Hôpital de la Pitié Salpêtrière, DMU de Neurosciences, Paris, France. (3) Service de Neurologie, CRMR SLA, CHU Dupuytren, Limoges, France.

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

To analyze potential dysfunctions of macrophages, we included FALS and SLAS as well as asymptomatic ALS mutation carriers and healthy controls. Monocytes from whole blood were differentiated *in vitro* into macrophages and then activated with pro- or anti-inflammatory stimuli. Immunological responses of monocytes/macrophages were studied at different levels: transcriptome, cells surface marker expression, and secretion profile.

Preliminary results show that, transcriptome and secretome of patients and controls were clustering in response to the stimuli, but we were also able to segregate groups of patients with different response profiles defined as “low and high responders”. Analyses showed that macrophages from patient with C9ORF72 expansion were mostly belonging to the “low responder” group. Those results were confirmed with a larger cohort of patients and compared to additional control groups including asymptomatic C9ORF72 expansion carriers and other non-inflammatory motor neuron diseases.

Our results could provide insight into the involvement of macrophages in ALS pathophysiology and a potential solution to segregate ALS patients with specific features to target disease progression. We

are currently investigating potential pathways to target in macrophages to impact disease progression as well as disease biomarkers.

Keywords: Macrophages, Biomarkers, Stratification.

Acknowledgement: We wanted to acknowledge patients and their families for participating to this study.
This work is financed by ARSLA, FRM EQU202103012581, ALSA, Ferbanc, l'ARMC, S.L.A.F.R., La longue route des malades de la SLA, Un pied devant l'autre, Invincible été, private donors to the ICM.

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OC 1.4 - Deciphering the crosstalk between motor neurons and microglia in Amyotrophic Lateral Sclerosis with human spinal cord organoids

Ghikh-Mimiette K (1), Liu E (1), Jost-Mousseau C (1), Le Goff G (1), Seilhean D (1), Millecamps S (1), Lobsiger CS (1), Boillée S (1), Bohl D (1)

(1) Institut du Cerveau - Paris Brain Institute - ICM, CNRS UMR 7225, Inserm U1127, Sorbonne Université UMR S 1127, AP-HP, Hôpital de la Pitié-Salpêtrière Paris (France)

The challenge in developing treatments for ALS stems from the heterogeneity of the disorder. Despite this complexity, there is a neuropathological signature common to almost all sporadic ALS and most familial ALS cases. The hallmarks consist of the specific degeneration of motor neurons (MNs), the accumulation of neurofilaments, the formation of Bunina Bodies, and both the nuclear clearance of TDP-43 and the presence of TDP-43 cytoplasmic inclusions. Inflammation is also a common signature observed in all ALS patients. Previous results from Dr Boillée's team showed that microglial cells were progressively activated with proinflammatory responses to MN degeneration and impacted ALS disease progression in mutant SOD1 mice models [1]. However, a significant limitation is the lack of human-relevant models able to reproduce the crucial disease hallmarks. The induced pluripotent stem cell (iPSC) technology could provide such a model, but we and others have shown that some ALS hallmarks are not fully reproduced in iPSC-derived MNs [2]. To go one step further and have a more integrated cellular model allowing MN interactions with their environment, we want to study pathological hallmark formation, MN degeneration and microglia contribution to these features in spinal cord organoids. For this purpose, iPSC from control subjects and ALS patients with various ALS forms were used to generate spinal cord organoids. Our analyses over 3 months of culture showed that iPSC in these organoids differentiated into MNs, interneurons and astrocytes. Additionally, we cultured organoids with microglia progenitors, showing their integration and maturation within the spheres. We are currently investigating the contribution of microglia to MN degeneration in these organoids generated with control and ALS iPSC using matched and mismatched conditions. This new human model for ALS could allow a better understanding of disease mechanisms and the discovery of novel pathways as targets for future therapies.

References: [1] Chiot, Aude, et al. Modifying macrophages at the periphery has the capacity to change microglial reactivity and to extend ALS survival. Nature Neuroscience, 2020; 23: 1339-1351 [2] Lefebvre-Omar, Cynthia, et al. Neurofilament accumulations in amyotrophic lateral sclerosis patients' motor neurons impair axonal initial segment integrity. Cellular and Molecular Life Sciences, 2023; 80: 150.

Key words: iPSC, Organoids, Microglia

Acknowledgements/funding: This work is funded by ANR LaBex Revive (ANR-10-LABX-73), FRM EQU202103012581, Neuratris, ARMC, S.L.A.F.R., La longue route des malades de la SLA, Un pied devant l'autre, Invincible été, Donateurs privés. ICM, INSERM.
Contacts: k.ghikh-mimiette@icm-institute.org; delphine.bohl@icm-institute.org

OC 1.5 - Unconventional secretion of misfolded SOD1 in ALS: a novel mechanism of toxicity spreading

Gosset P, Brugioti V, Néel E, Labatut M, Lappeman S, Ait Lahcen Y, Challuau D, Scamps F, Mezghrani A, Raoul C

The Institute for Neurosciences of Montpellier, Inserm UMR 1051, Univ Montpellier, Saint Eloi Hospital, Montpellier, France

Amyotrophic lateral sclerosis (ALS) is characterized by the selective loss of motoneurons leading to paralysis and death. Among the familial forms of the disease (10%), the first gene identified codes for an ubiquitous protein, superoxide dismutase type 1 (SOD1). Transgenic mice expressing mutated human forms of SOD1 faithfully summarize the main features of the disease. Spread of proteins from one region to another involving several cell type is proposed in ALS. Prion-like theory might explain spreading but is not well understood at cellular level. Our objective is to understand how ALS-causing protein can be secreted and be therapeutically targeted to stop disease progression. Through the description of unconventional secretion mediated by USP19, we study the secretion of $SOD1^{G93A}$ mutant using cell culture system and expression of functional mutants. The analyzis of UPS19 expression pattern is done at different disease stages by immunofluorescence and biochemistry in $SOD1^{G93A}$ mice. For the gene therapy development, several AAV serotypes through different route of administration in mice are tested to optimize delivery in oligodendrocyte. Micro-RNA-embedding small interfering sequences are validated to silence efficiently USP19 *in vitro*. Preclinical evaluation of viral-mediated silencing of USP19 includes behavior, histopathological and biochemical analysis. Our data show that the USP19 promotes the secretion of $SOD1^{G93A}$ initiating its loading at the endoplasmic reticulum. We found that USP19 is predominantly expressed in oligodendrocytes and show differential expression levels in ALS mice. The intracerebroventricular deliveries of an AAV allow us to target specifically oligodendrocytes in the spinal cord. The interferent RNA designed silence the expression of USP19 *in vitro*. The gene therapy in $SOD1^{G93A}$ mice reveals behavior and post-mortem abnormalities. These results provide new knowledge on the proteinopathy aspect and pave the way for the preclinical evaluation of a targeted intervention in ALS mice.

Keywords: Amyotrophic lateral sclerosis, SOD1, USP19

Acknowledgments: ARSLA fundings, ANR "SPREADALS" fundings.

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OC 1.6 - Contribution of neutrophils extracellular DNA traps to Amyotrophic Lateral Sclerosis pathogenesis

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(1) Institute for Neurosciences Montpellier, Inserm UMR 1298, University of Montpellier, France. (2) Department of Neurology, University Hospital of Montpellier Gui de Chauliac, France

Purpose: Today, there is strong evidence that chronic inflammation of the central nervous system (CNS) associated with dysregulation of the immune system is involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) [1]. A study of 1,030 ALS patients showed that a higher neutrophil/lymphocyte ratio was associated with a faster disease progression and had a negative effect on the survival [2]. However, the role of neutrophils in ALS has never been investigated. Neutrophils are the first line of defense against invading pathogens and initiate the inflammatory response. NETosis is one of the defense mechanisms of neutrophils. This process leads to the production of an extracellular network of DNA filaments and antimicrobial molecules called "Neutrophils extracellular DNA Trap" (NETs), whose purpose is to trap and eliminate infectious agents. Neutrophils and NETs have been implicated in the pathogenesis of several diseases including neurodegenerative disease [3]. Here, we first study the contribution of neutrophils and NETs in the pathophysiology of ALS.

Methods: Circulating neutrophils were characterized by flow cytometry analyses in ALS mouse model ($SOD1^{G93A}$) and ALS patients. Neutrophil infiltration and the presence of NETs in the spinal cord were observed by flow cytometry and immunofluorescence. *In vitro* NETs induction and their cytotoxicity toward motor neurons were evaluated with a Live-Cell Analyses System (Inucyte).

Results: We observed a higher neutrophil/lymphocyte ration and a higher proportion of a subpopulation of immunosuppressive neutrophils ($CD16^{high}CD62L^{dim}$) in ALS patients. We highlighted an infiltration of neutrophils and lymphocytes in the spinal cord of $SOD1^{G93A}$ mice. Neutrophils from ALS mice at the terminal stage of the disease are more prone to produce NETs *In vitro*. NETs seems to have an impact on motor neurons degeneration.

Conclusion: Neutrophils are part of immune cells that play a role in the chronic inflammation that occurs in ALS.

Reference:

[1] Vahsen, Björn F et al. “Non-neuronal cells in amyotrophic lateral sclerosis - from pathogenesis to biomarkers.” *Nature reviews. Neurology* vol. 17,6 (2021): 333-348.

[2] Wei, Qian-Qian et al. “Neutrophil-to-lymphocyte ratio in sporadic amyotrophic lateral sclerosis.” Neural regeneration research vol. 17,4 (2022): 875-880.

[3] Shafqat, Areez et al. “Neutrophil extracellular traps in central nervous system pathologies: A mini review.” Frontiers in medicine vol. 10 1083242. 17 Feb. (2023).

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

Acknowledgments: This project is supported by the Radala Foundation for ALS Research

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FILSLAN « TRAINING THROUGH RESEARCH » PROJECT AWARD 2023

- **Study of the pathogenicity of the TDP-43 protein in the neurodegenerative impairment of sporadic Amyotrophic Lateral Sclerosis**

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Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative disease in adults. Characterized by motor neurons degeneration, it is responsible for a progressive diffuse paralysis leading to death around 2 years after the onset of symptoms. To date, there is no curative treatment for ALS due to the lack of knowledge in the pathophysiological mechanisms involved and the absence of cellular and animal models that faithfully reproduce the disease. The TDP-43 protein was identified in 2006 as a central player in the pathophysiology of ALS which forms pathological aggregates in neurons of patients. Prion-like properties of TDP-43 have recently been described and seems to play a major role in the propagation of the disease. Based on these findings, we initiated the generation of a new mouse model by induction of TDP-43 insoluble extracts from ALS human brain. We hope that this approach will allow us to generate a mouse model representative of the sporadic pathology in humans, allowing us to improve our knowledge on the pathophysiology of ALS and open new therapeutic perspectives.

- **Epistemic challenges of amyotrophic lateral sclerosis (ALS)**

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Amyotrophic lateral sclerosis (ALS), better known in France as Charcot's disease, is a neuro-evolutionary disease that has been described in many ways: "the worst disease", "the cruelest disease", "the least rare disease", "the most brilliant discovery of Jean-Martin Charcot". These formulas give a unique status to this disease, which is still little studied by philosophy, unlike, for example, Alzheimer's disease, a neuro-evolutionary disease that is much more common. They were a catalyst for the philosophical inquiry that constitutes this work, which focuses on the epistemic challenges of ALS. Epistemic challenges are the theoretical and practical problems that can arise from a plurality of perspectives (or epistemic plurality) on the same object. They are challenges in the sense that the ALS community needs to address them in order to avoid possible harmful consequences. My thesis examines three of them in particular. In the first part, the idea that ALS is the exemplary disease in terms of medical difficulties in understanding and treating it is identified, analyzed, and challenged. It is shown that in approaching discourses on ALS it is important to take into account the distinction

between ALS as studied and characterized by medicine and biology (disease), ALS as lived experience (illness), and ALS as social phenomenon (sickness). The second part examines the historiography of ALS. It is shown that the focus on the genesis of the concept of ALS in Charcot's work may be a factor of ignorance for the history of medicine and for the current conception of ALS. The third part examines different categorizations of ALS: ALS as a disease or syndrome, as a motor disease and as a rare disease. Epistemological, practical, and ethical issues are highlighted in light of their relevance and limitations. By examining the discourses on ALS, my thesis aims to nuance and dialogue them in order to propose an overall reading grid for this complex subject. Addressing the epistemic challenges of ALS also serves to show the extent to which considering the concept of ALS as a 'boundary object' can be fruitful, including in philosophy, particularly in order to account for the conceptual fragmentation at work when it comes to the concept of disease.

Keywords: Charcot disease, ALS, neurology, disease, philosophy of medicine, applied philosophy, epistemic challenges, epistemic plurality, conceptual fragmentation, boundary object

- **Therapeutic effects of the borna virus x protein in SOD1^{G93A} mice**

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Among the pathophysiological hallmarks of amyotrophic lateral sclerosis (ALS), mitochondrial dysfunctions appear to be one of the earliest events and thus might be causative for the progressive loss of motoneurons (Le Masson et al., 2014). Therefore, restoring mitochondrial functions could be a therapeutic area of interest in developing new therapies against this disease.

For this purpose, we are interested in the Borna disease virus (BDV) X protein. When it targets the mitochondria, the X protein acts on the preservation of mitochondrial function and inhibits neuron apoptosis (Poenish et al., 2009). X protein has also been shown to protect neurons from degeneration in an animal model of Parkinson's disease (Szelechowski et al., 2014). Moreover, manipulation of the N-terminal sequence of the X protein to obtain XA4 protein enhances mitochondrial targeting and neuroprotective effect (Ferré et al., 2016).

Based on this knowledge, our study aim was to test the neuroprotective potential of both X and XA4 proteins in the SOD1G93A mice. AAVrh10 viral vector known to have efficient transduction in the spinal cord was used to administrate intra-cerebro-ventricularly either X or XA4 proteins to SOD1^{G93A} mice. Progression of motor deficits was then followed with motor tests (rotarod, grip test, and inverted grid). Motoneuron survival and integrity of neuro-muscular junctions were quantified by immunohistochemistry and the lifespan of the animals was determined.

Our results confirm the therapeutic power of the BDV X protein in SOD1^{G93A} mice, as previously shown in another study conducted in our lab. However, X and XA4 proteins do not have the same behavioral effects. On the one hand, the X protein delays motor impairment, while on the other hand, the XA4 protein increases the life expectancy of SOD1G93A mice.

Since both the X and XA4 proteins increase in the same way the number of lumbar motoneurons, the improvement in motor performance with the X protein is not due to better protection of motoneuron survival. However, a better and more significant preservation of neuromuscular junction was observed in mice treated with X protein compared to mice treated with XA4 protein.

In addition, the increase of lifespan observed in mice treated with the AAVrh10-X_{A4} vector is not explained by better preservation of phrenic motoneurons since quantification of retrogradely labeled neurons from diaphragm muscle was not different between AAVrh10-X and AAVrh10-X_{A4} treated mice. Further investigations are therefore required to identify the target of the X_{A4} protein involved in increasing animal survival.

SESSION 2: BIOMARKERS AND THERAPIES IN MOTOR NEURONE DISEASES

Moderation: Edor KABASHI, Philippe CORCIA

- **Guest lecture**

C2 Conference: Antisense oligonucleotide therapies for ALS

Philip VAN DAMME

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- **Presentations selected from abstracts submitted**

OC 2.1 - Identification of proteomic clusters in the CSF of sporadic ALS patients

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Introduction: ALS heterogeneity poses challenges in understanding and comprehensively treating the disease. Recent studies, including our own [1,2], demonstrated that ALS patients can be divided into different clusters according to their gene expression level in post-mortem tissue. Clustering based on cerebrospinal fluid (CSF) proteins might therefore hold great promise towards personalized medicine for ALS. **Method:** In this study, we conducted a proteomic analysis (label-free nanoLC-MS/MS) of CSF from 50 ALS patients and 52 controls. Four clustering approaches (hierarchical, model-based, k-means, and partitioning around medoids) were used to identify ALS clusters, and gene set enrichment analyses was performed on the differentially abundant proteins. **Results:** The ALS cohort consisted of 68% males, mean age of 65 years and 28% bulbar disease onset. Demographics were not significantly different from the control cohort. Clustering analyses identified two and three robust clusters, each exhibiting distinct pathway alteration patterns. Focusing on the two cluster results, one showed prominent upregulation of the immune and coagulation pathways, including immunoglobulins (IGKV4-1, IGHV1-69, IGHA2), complement activation proteins (C2/3, C5-9), and fibrinogens (FGA, FGB, FGG). The other cluster demonstrated an increased abundance of proteins involved in synaptic and cell junction/adhesion pathways, including members of the tyrosine protein phosphatase receptor family (PTPRN, PTPRS, PTPRZ1), members of the cadherin and collagen family (CDH6/2/8, Col6A2/3) and growth-associated proteins (GAP43, BASP1). When focusing on three clusters, the two previously

described clusters remained biologically conserved. However, the third cluster formed an in-between cluster with significant upregulation of proteins linked to the previously described pathways, but also associated with cell development and morphogenesis (CDH11, CXCL12, TNFRSF21). **Conclusion:** Our study demonstrated that ALS heterogeneity can partially be explained by distinct proteomic patterns in the CSF of ALS patients. Stratification by CSF clusters may open avenues for clustering in clinical trials to select efficacy-expected subpopulations.

References:

[1] Eshima J, O'Connor SA, Marschall E, et al., Molecular Subtypes of ALS Are Associated with Differences in Patient Prognosis, *Nat Commun* **2023**, *14*, 95. [2] Gomes LC, Hänzelmann S, Hausmann F, et al., Multiomic ALS Signatures Highlight Sex Differences and Molecular Subclusters and Identify the MAPK Pathway as Therapeutic Target, *bioRxiv* **2024.**

Keywords: Clustering, Proteomic, Biomarker.

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OC 2.2 - Sigma-1 receptor activation as a strategy to counteract ALS pathology

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Amyotrophic lateral sclerosis (ALS) is characterized by a degeneration of motor neurons, leading to muscle weakness and progressive paralysis. Currently, no cure is available to halt or reverse the progression of the disease. Oxidative stress, mitochondrial dysfunction, accumulation of unfolded proteins and inflammation are interconnected key actors involved in ALS. A potent therapeutic strategy would be to find molecules that break this vicious circle leading to neuronal dysfunction and death. Targeting Sigma-1 receptor (S1R) could meet this objective, as this chaperone protein modulates many cell survival mechanisms. So far, the impact of S1R activation was studied using specific agonists and mostly on mutant SOD1 models.

Our objectives are to determine how much S1R activation is protective in the ALS context and to better understand the mechanisms by which S1R acts. We compared the impact of two different S1R activators: the reference agonist PRE-084 and the positive modulator OZP002. Both S1R activators, significantly alleviated locomotor deficit of zebrafish models expressing mutations of TDP43 or C9orf72. More importantly, PRE-084 or OZP002 administration by intraperitoneal injection was able to ameliorate locomotor performances and coordination of $TDP43^{A315T}$ mouse model. In a previous study, our data suggested a possible role of the nuclear factor erythroid-derived 2 -like 2 (NRF2) pathway in the protective effects of S1R (1). NRF2 cascade is known to regulate oxidative stress and inflammation. We further investigated the role of this cascade and our results indicate that treatments of zebrafish with PRE-084 or OZP002 increase the levels of mRNA encoding NRF2 as well as some of its targets. Moreover, downregulation of NRF2 with antisense oligonucleotide prevented the protective

effect of both S1R activators on zebrafish models. Thus, we provided direct argument that S1R-induced beneficial effects are in part mediated by NRF2 pathway.

References: (1) Lasbleiz, C. et al. (2022) Sigma-1 receptor agonist PRE-084 confers protection against TAR DNA-binding protein-43 toxicity through NRF2 signalling. Redox Biol 58, 102542
Keywords: Tar DNA-binding protein 43 kDa (TDP43), C9orf72, Sigma-1 receptor
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OC 2.3 - Longitudinal MRI Study Reveals Potential Neurodevelopmental Disorders in *C9Orf72* Mutation Carriers

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Mutations in the *C9Orf72* gene are linked to many familial ALS and FTD cases. Recent studies (1, 2) suggest that *C9Orf72*-related pathology might originate during neurodevelopment. This raises questions about disease mechanisms and the identification for reliable biomarkers of degeneration for clinical trials.

Objective of this study was to describe longitudinal cervical spinal cord (SC) pathology using SC MRI in a cohort of asymptomatic *C9Orf72* first-degree relative subjects.

Methods: 72 asymptomatic individuals were enrolled in a prospective study of first-degree relatives of ALS and FTD patients carrying the *C9orf72* mutation. 40 (C9+) were carriers. Each subject underwent a 3T cervical SC MRI. Quantitative measures of GM and WM atrophy and DTI parameters were evaluated at baseline, after 18 and after 36 months.
Results: At baseline, significant WM atrophy was detected in C9+ subjects older than 40 years of age (p-value < 0.05) without associated changes in GM. At 18 and 36-month follow-up, no modification was observed both in WM and GM. Progressive significant fractional anisotropy (FA) reduction in the cortico-spinal tract was observed over the three time points in the C9+ group (p = 0.04).

Discussion: The cervical spinal cord imaging of *C9orf72* hexanucleotide carriers reveals age-related WM atrophy, which remains stable over time, and no GM atrophy. Pyramidal tract FA reduction is observed in C9+ subjects compared to C9- subjects and shows significant progression in follow-up scans. Stable WM atrophy might indicate a neurodevelopmental condition, which would explain the lack of progression over time, while the relation to increasing age of the subjects is similar to the one described in the general population. In contrast, changes in FA over time could be an early marker of

progressive degeneration before motoneurons in the GM begin to degenerate. Further studies with larger populations, including symptomatic C9+ subjects, are needed to confirm this hypothesis.

1- Lule DE et al. Deficits in verbal fluency in presymptomatic C9orf72 mutation gene carriers-a developmental disorder. J Neurol Neurosurg Psychiatry. 2020 ;91(11):1195-1200. doi: 10.1136/jnnp-2020-323671.
2- Hendricks E et al. The C9ORF72 repeat expansion alters neurodevelopment. Cell Rep. 2023; 42(8):112983. doi: 10.1016/j.celrep.2023.112983.

Keywords

C9Orf72, spinal cord MRI, neurodevelopment

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OC 2.4 - Brainstem as a proxy for respiratory and bulbar function in Amyotrophic Lateral Sclerosis

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Background:

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegeneration of motor neurons, which eventually leads to death mainly by respiratory failure. Respiration, speech, and swallowing functions are often compromised in ALS are thought to be related to not well characterized brainstem pathology. Notably, characterizing the pattern of brainstem atrophy among different ALS phenotype can provide valuable insights into the disease's pathophysiology and to be of utmost prognostic significance. Therefore, this study aims to investigate the differences in volumes of brainstem structures among ALS

phenotypes: bulbar, upper limb, and lower limb ALS. Additionally, it explores the correlation between these volumetric measurements and clinical measures of bulbar and respiratory function assessed by standardized tools.

Method:

This study included 202 ALS patients, shortly after receiving the diagnosis, from the large French cohort PULSE study (protocol 2013-A00969-36): 50 with bulbar onset, 78 with lower limb onset, and 74 with upper limb onset. Volumetric analysis of brainstem regions was performed using 3 tesla system T1-weighted images. In addition, clinical and spirometry assessments were collected at the time of inclusion.

Results:

Bulbar onset ALS exhibited significant atrophy in brainstem regions, including the midbrain, pons, and medulla oblongata, compared to upper and lower limb onset ALS. In addition, significant positive correlations were observed between brainstem region volumes and spirometry measurements, including forced vital capacity (%), slow vital capacity (l), FVC supine, peak expiratory flow, sniff nasal inspiratory pressure, and maximum inspiratory pressure. Furthermore, significant positive correlations were found between brainstem region volumes and the bulbar function assessed by ALSFRS-R score.

Conclusion:

These findings highlight the potential of quantifying brainstem atrophy as valuable marker that could reflect bulbar and respiratory function impairment in ALS, which is of utmost importance for clinical practice and trials.

Keywords: *Amyotrophic Lateral Sclerosis, MRI, Brainstem, Respiratory, bulbar, spirometry*

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OC 2.5 - Characterization of a therapeutic approach to deliver scFv targeting TDP-43 pathology in ALS

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A hallmark of ALS is the presence of cytoplasmic aggregates of the TAR DNA/RNA binding protein (TDP-43) in 97% of patients. The aim of our study is to develop biotherapeutics targeting TDP-43 proteinopathy.

Using phage display, we identified single chain variable fragment (scFv) clones (B1 and D7) exhibiting human wildtype(wt) TDP-43-specific affinity. We generated *in silico* a 3D model of full-length TDP-43 for epitope prediction, and verified the interaction of the scFvs with TDP-43 using ELISA and Surface Plasmon Resonance (K_D=3.1E-9). We tested the effect of the scFv on TDP-43 proteinopathy in cell lines overexpressing TDP-43. The intrabodies seemed to be mainly nuclear in the absence of pathological TDP-43 and cytoplasmic when TDP-43 is overexpressed. D7 decreased the level of the insoluble 35 kDa C-terminal fragment of TDP-43 via proteasomal degradation, while B1 decreased the TDP-43-associated activation of NF-κB. Both scFv reversed some TDP-43-induced metabolic alterations, particularly associated with the lipid metabolism.

To enhance the delivery of the scFv protein inside cells, we complexed them to PEGylated SPIONs. Different mass ratios of SPION to scFv were tested for their size, zeta potential, and scFv retention capacity. The formulations were not toxic to cells following 4 and 24 hours of treatment. Flow cytometry and western blot analyses confirmed the cellular internalization of the SPIONs and scFv, respectively.

In conclusion, we successfully developed two intrabodies specific to human wildtype TDP-43 and able to counteract different aspects of TDP-43 pathology. To our knowledge, this is the first time that scFv are complexed to SPIONs for targeted cell delivery. Future studies will test the effect of SPION-scFv complexes on TDP-43 pathology in both *in vitro* and *in vivo* ALS models, and will make use of the neuron-specific viral vector AAV-CAP-B10 to test the effect of the scFv on a TDP-43 mouse model of ALS.

Key words: ALS, TDP-43, scFv.

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OC 2.6 - Care pathway heterogeneity in Amyotrophic Lateral Sclerosis: effects of sex, age class and onset-site related symptoms

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Introduction: Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease associated with heterogeneous clinical manifestations and restricted to symptomatic care management strategies. Benefit and timing assessment of ALS clinical interventions remains challenging for clinicians, further exacerbated by disease heterogeneity and differing delays reported in literature. We propose an approach centered on ALS patients' care pathways to describe care strategy temporality and diversity considering sex, age class and disease onset site.

Method: We developed an identification algorithm of incident ALS patients in the French medico-administrative database (PMSI). We then compared median times before transition and dynamics of clinical interventions occurrence, involved in disease progression, through semi-Markovian and Accelerated Failure Time models at two distinct time windows. Twelve different patient profiles were defined according to sex, age group and detected symptoms indicating disease onset site, to model care pathway trajectories considering interactions.

Results: We identified 21,153 incident patients with ALS between 2013 and 2022 with a mean age of 67.7±13.1 years, male/female and spinal/bulbar ratios of 1.2 and 1.9, respectively. Only 36.5% of patients presented a care pathway including at least non-invasive ventilation (NIV), gastrostomy or tracheostomy. We demonstrated that sex, age class and onset site partially explain care pathway strategies. Notably, older age, bulbar onset site and being a woman drive early initiation of gastrostomy and spinal onset site is associated with delayed NIV start while tracheostomy, mainly considered for younger patients (<64 years), is rarely considered in care management. Alongside, we report extensively the patient profile-specific estimated median delay before clinical intervention initiation.

Conclusion: Care pathway strategy investigation is crucial in the context of rare diseases restricted to life-support care management to anticipate patient needs and foresee healthcare strategies. In conjunction with multi-state models, health claims data can provide a detailed analysis of care pathways which reveals disparities in ALS care management strategies depending on patient profiles and contributes to improved support strategy anticipation.

Keywords: Amyotrophic Lateral Sclerosis, multi-state analysis, healthcare systems data.

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ARSLA SESSION

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

OC A1 - Elucidating the role of the spinal sympathetic autonomous neurons in ALS

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Amyotrophic lateral sclerosis (ALS) is characterized by the selective degeneration and death of motor neurons. However, non-motor dysfunctions including autonomic impairments have been reported in ALS patients and ALS animal models. The autonomic nervous system, divided into the sympathetic (SANS), the parasympathetic (PANS) and the enteric nervous systems, is responsible for regulating involuntary body functions such as heartbeat, blood flow, breathing, as well as metabolic functions (glucose homeostasis, increased oxygen consumption…). The SANS regulates the flight-or-fight responses preparing the body to action while the PANS slows down bodily processes. Disrupted energy homeostasis has been reported during ALS progression with metabolic alterations detectable before the onset of motor symptoms. During motor activities, increased metabolic expenditure requires a functional coupling between the sympathetic and somatic motor systems. Although supraspinal structures play a crucial role in this adaptive process, we have recently shown that the activity of sympathetic preganglionic neurons (SPNs), the major SANS output, and the motoneurons (MNs) could be coupled by the pharmacological activation of an intraspinal network revealing an independent spinal coupling between these two neuronal subtypes. SPNs and MNs share the same embryonic origin as well as many genetic and molecular similarities including a cholinergic phenotype and axonal projections to postsynaptic targets localized outside the central nervous system. Moreover, the synaptic targets of SPNs, the sympathetic postsynaptic neurons projects to the connections between MNs and muscles, the neuromuscular junctions (NMJs) and play a crucial role in the function and stabilization of these synaptic connections as well as in muscle force generation. The sympathetic and somatic systems appear therefore anatomically and functionally coupled at both the spinal and muscular levels. As a consequence, the physiology and thus the pathophysiology of the MN-muscle couple could not be investigated without including a third partner in this duo: the SANS. In the context of ALS, the SANS and in particular the SPNs has been largely neglected. In the present study, we have explored the SANS all along ALS progression in the SOD1 mouse model of ALS at both the *in vivo* and *in vitro* levels with a main focus on SPN functioning and motor exercise using genomic, histological and biochemical approaches as well as telemetric recordings of cardiovascular parameters in freely moving mice.

OC A2 - Unravelling the genetic contribution to prognosis and endophenotypes in ALS using an extended cohort of patients (PULSE-WGS)

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ALS is characterized by the simultaneous degeneration of motor neurons and corticospinal neurons leading to death within 2 to 5 years after onset. This straightforward clinical definition of ALS however covers widely heterogeneous clinical situations, with variable age at onset, pace of clinical progression or associated endophenotypes. ALS is also highly heritable, and several of the genetic factors associated with ALS are known to modify prognosis of patients. The possible role of genetic factors in disease progression, survival or the ALS endophenotypes has not been addressed. The PULSE Cohort study has been constituted by all French reference ALS centers and supported by ARSLA to characterize the natural history of ALS patients through multi-parametric assessment of the 500 patients included. Our current project aims at providing an overview of the genetics of patients included in the PULSE cohort. This will be achieved through C9ORF72 gene scan, whole exome sequencing and high-density genotyping in all PULSE patients. This will allow us to characterize the genetic status of patients included in PULSE with known ALS and neurodegeneration related genes and to enable systematic genotype phenotype correlation to identify possible genetic disease modifiers in terms of prognosis and endophenotypes. This study will allow us to better characterize the genetic correlates of ALS progression and endophenotypes.

OC A3 - Functionnal analysis of genetic variants of unknown significance in *SOD1* gene in Amyotrophic Lateral Sclerosis

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The *SOD1* gene encoding superoxide dismutase was the first gene identified in ALS, and its mutation is present in around 15% of familial ALS patients and 2% of sporadic ALS patients in France. Over 200 variants have been identified in the *SOD1* gene to date. The identification of a pathogenic or probably pathogenic variant (class 5 or 4, ACMG) in an ALS patient enables to propose a therapy by administration of an antisense oligonucleotide targeting SOD1 (ASO Tofersen, Biogen). Several variants in *SOD1* are of unknown significance (VUS, class 3, ACGM), including 67 listed in the Clinvar database.

We performed *in vitro* functional analyses to study 15 of these VUS or class 4 variants identified in the laboratory of Tours University Hospital (LBMR Diagnostic génétique de la Sclérose latérale amyotrophique). The *SOD1* genes carrying these variants were generated by site-directed mutagenesis on a plasmid pF146 pSOD1WTAcGFP1 and expressed *in vitro* by transfection of HEK and NSC-34 cell lines. Several markers of neurodegeneration were studied, such as the formation of protein aggregates, the morphology of neurites and cell viability. The expression of 5 variants was associated with the presence of SOD1-positive cytoplasmic aggregates as observed by fluorescence microscopy, and confirmed by western blot analysis of soluble and insoluble protein fractions. Our current analyses also suggest for 4 variants a reduction in neurite size of differentiated NSC-34. For some of these variants, the *in vitro* analyses are currently complemented by SeaHorse assays. *In vivo* behavioral studies in zebrafish are also underway, thanks to a collaboration with INM Montpellier.

The short-term objective of our study is to reclassify certain VUSs or class 4 variants of SOD1 into pathogenic variants, which would establish a cause of pathogenicity in patients carrying these

particular variants. Similar work is currently performed on VUSs identified in other major genes in ALS, such as *TARDBP*.

Keyword : SOD1, variant, function, aggregation

Etude Varials – funding ARSLA

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OC A4 - Involvement of NUP50 in Amyotrophic Lateral Sclerosis

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We previously identified that genetic variants in a nucleoporin, NUP50, are associated to an increased risk of ALS[1]. NUP50 is a dynamic nucleoporin found both at nuclear pore complex and within the nucleoplasm. Present at the nuclear pore, NUP50 participates in nuclear trafficking by interacting with different proteins implicated in the nucleocytoplasmic transport machinery. In the nucleoplasm, NUP50 interacts with chromatin, is more associated with euchromatin than with heterochromatin, althoughits specific functions in this context remain unknown s.

While our results indicate that NUP50 loss of expression is associated with ALS [1], there is no information on the pathogenic role of the rare NUP50 variants identified.

To elucidate the molecular consequences of these variants, we characterized the interactome of three of them: NUP50 R45C, Y156C and K275E. Our results show that these variants differentially alter the NUP50 interactome, with R45C showing profound effects, and the other two variants more restricted alterations in the network of protein interactions of NUP50. Furthermore, we show that expression of these variants in zebrafish leads to motor neuron defects. These findings suggest that NUP50 rare variants are pathogenic and affect both the NUP50 interactome and motor neuron survival and function.

[1] Megat S *et al.*, Integrative genetic analysis illuminates ALS heritability and identifies risk genes. Nat Commun. (2023) 14(1):342

Keywords: NUP50 – interactome- nucleocytoplasmic trafficking

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OC A5 - SOD1^{G93A} neurotoxicity initiated by subversion of cellular prion protein signaling in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most common motor neuron degenerative disease in adults. ALS is associated with the selective loss of motor neurons in the spinal cord, brainstem, and cortex, which causes progressive paralysis and death by respiratory failure. ALS belongs to proteinopathies, a family of diseases provoked by protein misfolding and dysfunction. Among other genes, the gene encoding Cu/Zn Superoxide Dismutase 1 (SOD1), an intracellular enzyme implicated in antioxidant processes, is frequently involved in ALS. The toxicity of SOD1 mutants relies on a toxic gain-of-function due to the misfolding, mislocalization, and formation of intracellular inclusions and aggregates of SOD1. The secretion and accumulation of ALS-linked SOD1 mutants in the surrounding milieu of motor neurons raises the unexplored possibility that abnormal SOD1 also triggers neurotoxicity through binding to membrane receptors and alteration of their signaling activity. Exploiting several neuronal cell lines exposed to misfolded human G93A SOD1 (hSOD1^{G93A}) and primary cultures of mouse motor neurons isolated from Tg-hSOD1^{G93A} transgenic ALS mice (referred to as ALS neurons), we show that plasma membrane cellular prion protein (PrP^{C}), a protein well-known for its implication in prion and Alzheimer's diseases, is a neuronal receptor for misfolded hSOD1^{G93A}. hSOD1^{G93A} interaction with PrP^{C} corrupts PrP^{C} signaling function, leading to 3-phosphoinositide-dependent kinase 1 (PDK1) overactivation and downstream under-shedding of plasma membrane TNFα receptors (TNFRs) by ADAM10/17 α-secretases. Cell surface accumulation of TNFRs renders ALS neurons hyper-vulnerable to TNFα inflammation. Infusion of a PDK1 inhibitor by the intraperitoneal route in Tg-hSOD1^{G93A} ALS mice rescues TNFR shedding mediated by ADAMs, protects spinal cord motor neurons from neurodegeneration, improves motor performance, and extends survival. Our work highlights that excessive stimulation of PrP^{C} coupling to PDK1 by hSOD1^{G93A} contributes to ALS and posits PDK1 as a novel target for developing therapeutics to mitigate ALS.

Keywords: Amyotrophic Lateral Sclerosis, PDK1, therapeutic target

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OC A6 - Anxa2 mRNA, missing its SMN partner, shows early defects of axonal localization in SMA motoneurons

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Spinal Muscular Atrophy (SMA) is a rare motoneuron (MN) disease, yet the most common genetic cause of infantile death. SMA is characterized by spinal cord MN degeneration, muscle atrophy and neuromuscular junction disruption. Deletions/mutations in the *Survival of Motor Neuron 1* gene leads to the production of a low level of full-length SMN protein. Due to a specific MN function of SMN in transporting several mRNAs in axons, a lower level of SMN in SMA might impair axonal mRNA transport and local translation, leading to a specific MN vulnerability. Our team has shown that Anxa2 mRNA localization in neurite is altered upon SMN depletion in a mouse cell line[1]. A motif in the mRNA, necessary for its neurite localization, was also identified[2]. Annexin A2 protein create bridges between membranes and microtubules, and is also concentrated in growth cones and branching, which suggests its significance in axonal outgrowth and cytoskeleton organization. Here, human iPSCs from SMA or healthy individuals were differentiated into MNs to study Anxa2 mRNA localization during axonal development. In all cell lines, Anxa2 mRNA axonal level decreased over time, and was always lower in SMA MNs during all the MN development compared to healthy axons. Immunoprecipitation of SMN and RNA-Protein Proximity Ligation Assay revealed an association of SMN and Anxa2, also higher in early development. SMN overexpression led to the rescue of Anxa2 mRNA in axons only when SMN was increased early on. Altogether, these results show that the axonal partnership between Anxa2 mRNA and SMN is occurring in early stages of MN development. This suggests a temporal role of this mRNA localization and its protein local translation at the beginning of axon development. In SMA, Anxa2 mRNA mislocalization might add to all the other defects described in the literature, to explain the specific vulnerability of MN.

[1] F. Rage *et al.*, "Genome-wide identification of mRNAs associated with the protein SMN whose depletion decreases their axonal localization," *RNA*, vol. 19, no. 12, pp. 1755–1766, 2013.

[2] K. Rihan *et al.*, "A new cis-acting motif is required for the axonal SMN-dependent Anxa2 mRNA localization," *RNA*, vol. 23, no. 6, pp. 899–909, Jun. 2017.

Keywords: SMA, axonal localization

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SESSION 3: MOLECULAR MECHANISMS OF MOTOR NEURONE DISEASES

Moderation: Philippe CODRON, Pascal LEBLANC

- ***Guest lecture***

C3 Conference: Understanding the life cycle of TDP-43: aggregation triggers & toxicity effectors

Magdalini POLYMENIDOU

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- **Presentations selected from abstracts submitted**

OC 3.1 - Propagation and toxicity of superoxide dismutase in Amyotrophic Lateral Sclerosis using human iPSC-derived motor neurons

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In ALS patients, the disease onset is often anatomically localized and can spread, to affect contiguous regions. One major pathological hallmark is the accumulation of misfolded proteins within motor neurons (MNs), which can adversely impact MN survival. To assist the conventional degradation pathways in managing the overwhelming amounts of misfolded proteins, the unconventional secretory pathway known as Misfolded Associated Protein Secretion (MAPS) dependent on the USP19 deubiquitinase, could be implicated. This pathway redirects misfolded proteins from degradation by removing ubiquitin residues, allowing them to form complexes with DNAJC5 chaperones, to translocate to late endosomes, and to potentially be secreted and taken up by surrounding cells. Analyzing whether this pathway is involved in the secretion and propagation of ALS misfolded proteins could enhance our understanding of disease spreading.

To address this, we generated MNs from four patients carrying different mutations in the *SOD1* gene, as well and isogenic and unrelated control iPSC clones. Our study first showed that misfolded SOD1 proteins (misSOD1) accumulated in mutant MNs compared to controls. Additionally, SOD1 proteins were secreted by MNs, and this secretion did not occur via the ER-Golgi or exosome pathways.

Focusing on the MAPS pathway, we found that many misSOD1 proteins co-localized with the DNAJC5 chaperone. Preliminary filter trap assays suggest than misSOD1 is secreted by mutant MNs. To determine whether this secretion was USP19-dependent, we used lentiviral vectors to overexpress or downregulate USP19 in MNs. While USP19 overexpression did not alter the colocalizations between misSOD1 and DNAJC5, its downregulation reduced these colocalizations, suggesting an involvement of the MAPS pathway in the secretion of misSOD1. Current experiments include co-cultures between control and mutant MNs to explore the possible USP19-dependent propagation of misSOD1 between

MNs. In conclusion, this study could identify a secretory pathway involved in the propagation of ALS pathological determinants.

Keywords: iPSC, USP19, misfolded proteins.

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OC 3.2 - Nifuroxazide rescues the deleterious effects associated with micos instability in disease models

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The identification of a point mutation (p.Ser59Leu) in the *CHCHD10* gene was the first genetic evidence that mitochondrial dysfunction can trigger motor neuron disease [1]. Since then, we have shown that this mutation leads to the disorganization of the MItochondrial contact site and Cristae Organizing System (MICOS) complex that maintains the mitochondrial cristae structure [2]. Here, we generated yeast mutant strains mimicking MICOS instability and used them to test the ability of more than 1600 compounds from 2 repurposed libraries to rescue the growth defect of those cells. Among the hits identified, we selected nifuroxazide, a broad-spectrum antibacterial molecule [3]. We show that nifuroxazide rescues mitochondrial network fragmentation and cristae abnormalities in *CHCHD10*$^{S59L/+}$ patient fibroblasts. This molecule also decreases caspase-dependent death of human *CHCHD10*$^{S59L/+}$ iPSC-derived motor neurons. Its benefits involve KIF5B-mediated mitochondrial transport enhancement, evidenced by increased axonal movement and syntaphilin degradation in patient-derived motor neurons. Our findings strengthen the MICOS-mitochondrial transport connection. Nifuroxazide and analogues emerge as potential therapeutics for MICOS-related disorders like motor neuron disease. Its impact on syntaphilin hints at broader neurological disorder applicability for nifuroxazide.

[1] : Bannwarth Sylvie, Samira Ait-El-Mkadem, Annabelle Chaussenot et al. « A Mitochondrial Origin for Frontotemporal Dementia and Amyotrophic Lateral Sclerosis through CHCHD10 Involvement ». *Brain* 137, n° 8 (août 2014): 2329-45. https://doi.org/10.1093/brain/awu138.

[2] : Genin Emmanuelle C, Morgane Plutino, Sylvie Bannwarth et al. « *CHCHD 10* Mutations Promote Loss of Mitochondrial Cristae Junctions with Impaired Mitochondrial Genome Maintenance and Inhibition

of Apoptosis ». *EMBO Molecular Medicine* 8, n° 1 (janvier 2016): 58-72. https://doi.org/10.15252/emmm.201505496.

[3] : Althagafy Hanan S., Mostafa K. Abd El-Aziz, Islam M. Ibrahim et al. « Pharmacological updates of nifuroxazide: Promising preclinical effects and the underlying molecular mechanisms ». *European Journal of Pharmacology* 951 (15 juillet 2023): 175776. https://doi.org/10.1016/j.ejphar.2023.175776.

Keywords : ALS, mitochondrial-disease, nifuroxazide
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OC 3.3 - Automated analysis of iPSC-derived motor neurons from TARDBP ALS patients reveals altered neuronal net organization and subcellular TDP43 localization

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Like other neurodegenerative diseases, the degenerating neurons of ALS patients are characterized by the accumulation of cytoplasmic aggregates enriched in ubiquitinated TDP-43. Expressed mainly in the nucleus and partially in the cytoplasm, the exact mechanisms of TDP43 toxic aggregation in motor neurons have not yet been described, but mutations in the TARDBP gene (encoding for TDP-43 protein) have been associated with ALS pathogenesis. Induced pluripotent stem cell (iPSC)-derived motor neurons offer a promising platform for studying ALS pathogenesis, however, current methodologies for analysing iPSC-derived motor neurons are often time-consuming, and subject to inter-experimental variability, limiting their use for large-scale and drug screening studies. Moreover, the study of ALS-derived motor neurons has showed big discrepancies in the observed phenotypes between studies and research groups, which need to be addressed. In our study, we aimed to address these limitations by developing automated assays specifically for the robust and reproducible analysis of iPSC-derived motor neurons from ALS patients with TARDBP mutations. Our automated assays encompassed several crucial aspects of motor neuron pathology that include neurite outgrowth and neuronal population organization, survival assays, electrophysiology experiments, as well live automated confocal imaging of subcellular components. Using these assays, we were able to show that TARDBP motor neurons have higher neurite length compared to control lines, associated with a more disorganized neuronal network. Moreover, patient TARDBP lines had an increased mortality rate during their maturation phase. The subcellular localisation of TDP43 was also altered in patient lines, with TDP43 localizing slightly more in the cytoplasm, and more specifically near and inside the mitochondria. In conclusion, automating the analysis of these phenotypic features allowed us to detect very light changes that could have been missed by a more classical analysis approach. The next step will be to use the developed assays for drug screening experiments to alleviate the observed phenotypes.
Keywords: iPSC-derived motor neurons - TDP43 – mitochondria
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OC 3.4 - Towards Understanding C9orf72 Repeat Instability in ALS/FTD

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are often caused by expanded G_4C_2 hexanucleotide repeats in the first exon/promoter region of the C9orf72 (C9) gene. The expanded G_4C_2 repeats are highly unstable, both intergenerationnally and between different types tissues, organs and cells of individual patients. It remains a huge challenge to correctly diagnose C9 repeat size in blood and to understand the mechanisms of repeat instability in brain.

To address these challenges, we set up a novel technique of optical C9 repeat sizing (Bionano) and evaluated its sensitivity and precision using C9 BAC plasmids, IPSC lines and lymphoblastoid cell lines (LCLs). We found that optical C9 repeat sizing correctly determined repeat size in BAC plasmids carrying 4, 55 or 857 repeats. Furthermore, C9 repeat sizes of normal and pathologically expanded C9 alleles in IPSC and LCL determined by our novel technique were closely correlated to repeat sizes determined by Southern blot.

To evaluate the diagnostic potential of optical C9 repeat sizing, we analyzed blood samples from 15 C9 ALS/FTD cases and C9 mutation carriers. We found that optical C9 repeat sizing was able to resolve the full spectrum of pathologically expanded C9 alleles including short alleles of 37 repeats and long alleles exceeding 6.000 repeats.

To analyze repeat instability in brain, we compared C9 repeat sizes between different regions of human postmortem C9 ALS and FTD brains. C9 repeats in motor cortex, frontal cortex and brainstem seemed longer and extended over a wider range than C9 repeats in cerebellum. To understand this difference, we started to analyze C9 repeat profiles in various cell types such as neurons, astrocytes, oligodendrocytes and microglia of C9 ALS/FTD cases.

To begin to unravel the mechanisms of C9 repeat instability in brain, we generated human C9 IPSC lines from two C9 ALS patients and one mutation carrier and differentiated the IPSCs into forebrain cerebral organoids (COs). Immunohistochemical analysis of COs at 64 days indicated the presence of SOX2+ pluripotent stem cells, Nestin+ neural stem cells as well as TUBB3+ and MAP2+ neurons. Optical repeat sizing demonstrated that C9 COs contain a wide range of repeat expansions and contractions, mimicking the situation in adult human brain. By contrast, the C9 IPSC lines displayed only a single pathological C9 allele. Strikingly, C9 repeat sizes from mutation carriers were stable from day 0 to days 64 and 150. Taken together, these data suggest that premutation C9 repeats are stable during neural development whereas pathologically expanded repeats become unstable during early phases of neural development.

In conclusion, optical C9 repeat sizing holds promise to improve the diagnosis of C9-linked ALS/FTD, to perform genotype/phenotype studies and to better understand the mechanisms of C9 repeat instability.

Keywords: C9orf72, C9 repeat instability, optical mapping, forebrain cerebral organoids

Acknowledgments: We gratefully acknowledge funding of this work by Association de Recherche sur la SLA (ARSLA). Additional funding was obtained from Association Française contre les Myopathies (AFM), Agence Nationale pour la Recherche (ANR) through the eRARE3 program and Région Ile de France through the Sesame 2019 equipment grant.

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OC 3.5 - Beneficial effect of the environmental enrichment on a transgenic mouse model of FTD-ALS (FUSΔNLS+/-)

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

The heterozygous FUSΔNLS mouse model expresses a FUS truncated protein without Nuclear Localization Signal. Mice were housed in EE (Marlau cages, n=10-12/cage) versus Standard Environment (SE, n=2/cage), for 4 months after weaning until the behavioral tests (at five months of age). A parallel cohort was raised for RNAseq and epigenomic (Cut&Tag-seq) experiment of the hippocampus.

We found that in the MWM task, FUS EE mice showed a significantly better performance compared to FUS SE. In the OIP task FUS EE mice were significantly better than SE mice. Transcriptomic data revealed that EE had a common effect of both FUS and WT mice by activating Immediate early genes as well as *Bdnf*. By contrast, multiple pathways related to neuronal/synaptic plasticity were dysregulated by EE only in FUS mice. At the epigenomic level, EE increased acetylation of H3K27 and trimethylation of H3K4 at synaptic and neurogenesis genes, some of them being modulated by both marks. Interestingly, the expression of associated genes was increased in FUS mice by EE, suggesting epigenetic regulation of these genes by EE in FUS mice.

Our findings demonstrate a beneficial effect of EE housing on FUS mice at the behavioral level. At the transcriptomic and epigenomic level, EE increased neuronal activity-associated gene transcription in both genotypes, but selectively impacted neuronal/synaptic plasticity genes in the FUS hippocampi.

Keywords: Enriched environment, Behavior, transcriptomics & epigenomics

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OC 3.6 - Role of FUS in Nucleolar Homeostasis

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Nucleolar homeostasis is essential for numerous cellular activities and overall cellular health. However, the key molecules that regulate nucleolar homeostasis remain largely unidentified. Recently, our team discovered that SMN plays a role in maintaining nucleolar homeostasis following DNA repair [1]. Our research aims to identify SMN interacting factors that could play a role in nucleolar homeostasis and gain a deeper understanding of their exact function in this process. Notably, FUS has been reported to interact with SMN [2], and mutations in FUS are known to cause amyotrophic lateral sclerosis (ALS) [3], making this protein an interesting candidate to study.

Our results revealed that FUS is retained at the nucleolar periphery following DNA damage induction and RNAP2 transcription inhibition. Preliminary findings from siFUS cells and doxycycline-inducible shFUS stable cell lines indicate that knocking down FUS disrupts nucleolar homeostasis after DNA repair. Furthermore, FUS knockdown reduces the retention of critical proteins, such as COILIN and SMN, at the nucleolar periphery, both of which are essential for nucleolar homeostasis post-DNA repair. Additionally, in both siFUS and shFUS cells, we observed a decrease in the levels of symmetrically dimethylated SMD1 protein, a component of the SR spliceosome, mirroring the results seen in shSMN cells.

In conclusion, we discovered that FUS is crucial for maintaining nucleolar homeostasis following DNA repair. Based on the evidence gathered, we propose the following hypothesis: FUS is involved in a specific type of RNA splicing at the nucleolar periphery, which aids in restoring nucleolar homeostasis after DNA damage-induced nucleolar stress.

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Key words: Nucleolar homeostasis, RNA splicing, FUS;

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C4 CONFERENCE

Moderation: Luc DUPUIS

Unlocking the Secrets of Minipuberty and GnRH Neurons: From Brain Development to Cognitive Resilience and Beyond

Vincent PREVOT

Development and plasticity of the neuroendocrine brain - INSERM University of Lille - CHRU Lille

ROUND TABLE: WHAT DID WE LEARN FROM RECENT FAILURES IN THERAPEUTIC TRIALS IN ALS

Moderation: Claire GUISSART, David DEVOS

Theme situation:

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

- **RT1 - Recent failures in ALS therapeutic trials, from a clinician's perspective: the challenge of clinical heterogeneity**
  Gaelle BRUNETEAU
  Neurology Department, Paris ALS Expert Center, Alliance on Clinical Trials for ALS-MND, Paris Brain Institute (ACT4ALS-MND, ICM), Myology Center For Research, Sorbonne University

- **RT 2 - Learning from failed trials in the past and proposals for the future**
  Thomas MEYER
  Charité – Universitätsmedizin Berlin, Center for ALS and other motor neuron diseases

- **RT 3 - Statistical learnings from recent therapeutic trials in ALS**
  Ruben VAN EIJK
  University Medical Center Utrecht

POSTER SESSION

P1: The new missense G376V-TDP-43 variant induces late-onset distal myopathy but not ALS

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TDP-43-positive inclusions in neurons are a hallmark of several neurodegenerative diseases including familial amyotrophic lateral sclerosis (fALS) caused by pathogenic *TARDBP* variants as well as more common non-Mendelian sporadic ALS (sALS). Here we report a G376V-TDP-43 missense variant in the C-terminal prion-like domain of the protein in two French families affected by an autosomal dominant myopathy but not fulfilling diagnostic criteria for ALS. Patients from both families presented with progressive weakness and atrophy of distal muscles, starting in their 5th-7th decade. Muscle biopsies revealed a degenerative myopathy characterized by accumulation of rimmed (autophagic) vacuoles, disruption of sarcomere integrity and severe myofibrillar disorganization. The G376V variant altered a highly conserved amino acid residue and was absent in databases on human genome variation. Variant pathogenicity was supported by *in silico* analyses and functional studies. The G376V mutant increased the formation of cytoplasmic TDP-43 condensates in cell culture models, promoted assembly into high molecular weight oligomers and aggregates *in vitro,* and altered morphology of TDP-43 condensates arising from phase separation. Moreover, the variant led to the formation of cytoplasmic TDP-43 condensates in patient-derived myoblasts and induced abnormal mRNA splicing in patient muscle tissue. The identification of individuals with TDP-43-related myopathy but not ALS implies that *TARDBP* missense variants may have more pleiotropic effects than previously anticipated and support a primary role for TDP-43 in skeletal muscle pathophysiology. We propose to include *TARDBP* screening in the genetic work-up of patients with late-onset distal myopathy. Further research is warranted to examine the precise pathogenic mechanisms of *TARDBP* variants causing either a neurodegenerative or myopathic phenotype.

Keywords: TDP-43, distal myopathy, protein aggregation

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P2: Neuropathological analysis of an ALS patient carrying a SOD1 missense mutation and a C9orf72 repeat expansion

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Typical neuropathology in Amyotrophic Lateral Sclerosis (ALS) patients with *C9orf72* mutation is widespread TDP-43-positive neuronal and glial cytoplasmic inclusions, together with p62-positive TDP-43-negative dipeptide repeat (DPR) proteins neuronal cytoplasmic inclusions (NCI) in the CNS. In *SOD1* mutations, those inclusions are absent, but SOD1 protein accumulates in the motor neurons, sparing the cortex. In this study, we present the neuropathological analysis of a clinically diagnosed ALS patient lacking familial history of ALS, carrying both p.Thr55Ileu SOD1 mutation and *C9orf72* repeat expansion.

A-63-year-old man initially presented gait disturbance. Six months later, he developed distal atrophy of the upper and lower limbs, apraxia, dysphagia, bilateral Babinski's sign and mild cognitive impairment. He died 15 months after onset. Post-mortem examination confirmed typical features of ALS, including severe loss of motor neurons and astrogliosis in the anterior horn of the spinal cord and hypoglossal nucleus. Cerebral atrophy was mild, preserving Betz cells.

TDP-43-positive NCI were noted in the anterior horn of the spinal cord, fronto-temporal cortex, motor cortex, hippocampus, and pontine nuclei. In addition, p62 immunohistochemistry revealed numerous inclusions in the cerebellar granule cells, known as the pathological hallmark of ALS/FTLD cases with *C9orf72* mutation. Anti-poly-GA immunohistochemistry showed abundant DPR inclusions in the cerebellum, motor cortex and hippocampus. The neuropathology was typical of a *C9orf72* mutation. In any region examined, no SOD1 pathology accumulation was found except rare swollen axons, labelled with anti-ubiquitin, -SOD1 and -neurofilaments in the spinal cord.

Dot Blot experiments are in progress to reveal the protein levels of native/misfolded SOD1 and DPR in this patient tissue compared to SOD1-, C9orf72-linked and other ALS patients without specific mutation in these genes.

These results are important in the context of SOD1-targeted gene therapy using antisense oligonucleotides, which may not be beneficial to such SOD1-linked patients, without SOD1 protein accumulation in neuronal cell bodies.

Key words: SOD1, C9orf72, double mutations

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P3: Secretion of misfolded TDP-43 mediated by the USP19 ubiquitin specific peptidase pathway

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Little is known about the mechanisms by which free TDP-43 aggregates can be secreted. Recently, a new unconventional secretion pathway mediated by the endoplasmic reticulum resident-deubiquitinase USP19 was identified in the release of the misfolded α-synuclein proteins.
We investigated the role of USP19 on the release of the misfolded K263E-TDP-43, an ALS associated mutant prone for aggregation. We observed a strong secretion of the K263E-TDP-43 upon the ER-USP19 overexpression. Conversely, the non-ER-associated USP19ΔTM or its ER-associated but deubiquitinase-inactive mutants failed to induce this release thus indicating that ER association and deubiquitinase activity are both essential. Characterization of conditioned media from USP19-WT/K263E-TDP-43 co-expressing cells through fractionation gradients and immunogold electron microscopy revealed that K263E-TDP-43 was mainly released as free aggregates. Interestingly, a significant increase of lipidated-LC3 was also observed in the USP19-WT co-expressing cells thus suggesting a role of the autophagic pathway. Inhibition of fusion between autophagosomes and lysosomes or inactivation of lysosomal activity using chloroquine or Bafilomycin-A1 inhibitors failed to impair aggregates release thus suggesting that lysosomal compartments are not essential. Conversely, inhibition of Vps34 using PI3-Kinase inhibitors or silencing of Atg7, Atg16L1, or Rab11A all involved in autophagosomes biogenesis, significantly inhibit the release of TDP-43 aggregates thus suggesting a role of the early autophagic compartments. Because autophagosomes can fuse with late endosomes to form secretory amphisomal compartments, we also investigated their implication by silencing HRS/HGS, a key ESCRT machinery component involved in their biogenesis. Our data revealed that silencing of HRS/HGS significantly reduces the release of misfolded TDP-43.
Trafficking and fusion of these compartments with the plasma membrane for the final secretion process can be mediated by different cellular factors including Rab8A and Rab27a or the v-SNARE Vamp7. Our data revealed that blocking these factors strongly reduces the release of TDP-43 aggregates.
This study points out a new potential pathway by which pathological TDP-43 could be released and disseminated.

Keywords : Misfolded TDP-43, Secretion, Deubiquitinase/USP19

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P4: Metabolomics of basal tears in Amyotrophic Lateral Sclerosis: a prospective comparative study

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BACKGROUND: Amyotrophic lateral sclerosis (ALS) is an incurable condition affecting motor neurons. The disease's clinical variability and the lack of conclusive diagnostic instruments result in average diagnosis delays of 9 months. The field of tear metabolomics emerges as a promising biomarker search strategy, particularly with advancements in analysing small-volume samples.

OBJECTIVE: Our objective was to assess whether metabolomic profiling of basal tears in ALS patients could act as a biological marker for diagnosing ALS, predicting prognosis, and discriminating between endophenotypes.

METHODS: A single-centre prospective case-control study was conducted in France from September 2021 to March 2023 including patients with ALS according to the revised EI Escorial criteria. Basal tears were pooled from both eyes using microcapillary glass tubes. A 2µL volume was analysed for every patient using ultra-high performance chromatography coupled with mass spectrometry. We performed univariate and multivariate analyses to determine if the metabolic signature could help discriminate ALS from controls and bulbar from spinal forms.

RESULTS: Twenty-five patients with ALS and 30 controls were included. No significant differences in metabolite levels were found between ALS and control groups ($p>0.05$). Basal tears metabolome significantly discriminated bulbar and spinal forms of ALS based on 6 metabolites, among which 5 were decreased (aniline, trigonelline, caffeine, theophylline, methyl beta-D-galactoside) in the bulbar form and 1 was decreased in the spinal form (dodecanedioic acid).

CONCLUSION: This study represents the first prospective analysis of basal tear metabolomics in individuals with ALS. Despite the inability to distinguish between ALS patients and controls based on metabolic signatures, these findings could contribute to understanding the phenotypic diversity of ALS. Notably, distinct metabolic profiles were identified that differentiate between the bulbar and spinal

forms of the disease. The metabolomics of basal tears might offer a valuable approach to uncovering significant biomarkers in neurodegenerative diseases.

KEYWORDS: amyotrophic lateral sclerosis; tears; metabolomics

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P5: Identification of specific molecular markers of ALS vulnerable motoneurons

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Modification of electrical activity of motoneurons is a key factor in amyotrophic lateral sclerosis (ALS) disease progression. Experimental evidence revealed a motoneuron-type vulnerability in ALS, beginning with the low excitability fast fatigable (FF) motoneurons, while the high excitability slow (S) motoneurons are preserved. These observations have led to the hypothesis that the high task demand of the FF motoneurons is responsible for their highest vulnerability. To broaden our understanding of the role of excitability in the selective degeneration and to improve the functional characterization of motoneurons types, we used the patch-seq method on motoneurons subtypes FF and S identified by patch-clamp electrophysiology [1] [2].

The expression of voltage-gated channels was analyzed in six FF motoneurons RNA banks and six S motoneurons RNA banks. *Cacna2d3*, a gene coding for $Ca_V\alpha2\delta3$, a regulatory subunit of high voltage activated calcium channels, was significantly increased in the FF motoneurons. Due to its high expression in ALS vulnerable motoneurons, we are currently investigating its role in motoneuron physiology and under ALS pathological condition.

In *Cacna2d3*$^{-/-}$ motoneurons, we show a drastic change in the subcellular localization of the P/Q type calcium channel $Ca_V2.1$, the major channel involved in spinal neurotransmission, implying a regulation of their function by the $Ca_V\alpha2\delta3$ subunit. In agreement with this results, behavioral studies show an increased endurance to locomotor task in the knocked-out mice.

The functional significance of Cacna2d3 in neurotransmission and firing properties of motoneurons will next be addressed with electrophysiological studies, as well as its impact on ALS progression by crossing the knock-out mice with a *SOD1*^{G93A} mouse line.

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Keywords: Motoneuron vulnerability, Calcium channel, Electrical activity

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P6: Deep cerebellar nuclei circuitry and connectivity in Amyotrophic Lateral Sclerosis (ALS)

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The cerebellum is best known for its motor functions, but there is growing evidence that it also contributes to many non-motor functions, such as cognition, emotional regulation and social functions. Motor and non-motor functions are supported by dense projections between the deep cerebellar nuclei (DCN), which represent the only output from the cerebellum, to multiple regions of the nervous system, including the spinal cord, red nucleus and thalamus. While the role of DCN in cerebellar function is well known, their connectivity and circuitry dedicated to their multiple functions remain to be elucidated. Using viral tracing experiments combined with intersectional mouse genetics, recent data identified direct DCN-spinal tracks that target local inhibitory V1 segmental neurons required for skilled movement and locomotion [1]. V1 interneurons innervate and make synapses directly on the soma of motoneurons, and they are affected at early stages of motor neurodegenerative diseases as in Amyotrophic Lateral Sclerosis (ALS) [2]. ALS is characterized by progressive paralysis, and V1 interneuron degeneration appears before motoneurons death. Loss of V1 interneurons appears as a potential key element to explore the non-autonomous vulnerability of motoneurons in the disease. Using the ALS $SOD1^{G93A}$ mouse model, we hypothesize that DCN circuits that directly innervate V1 interneurons may be affected during cerebellar development. Our hypothesis is sustained by our preliminary data that identified inhibitory synapses dysregulation in DCN neurons prior to the onset of ALS symptoms. Our aim is to identify the cellular and molecular mechanisms that alter DCN inhibitory synapse formation and organization. In addition, we will explore how these changes in inhibitory synapses affect the direct or functional connectivity of DCN to V1 interneurons, leading to their progressive loss.

Keywords : deep cerebellar nuclei, circuitry, synapse

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P7: Multimodal MRI analysis with machine learning for ALS biomarker discovery

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder affecting both upper and lower motor neurons characterized by considerable variability in site of onset and progression rate

among patients [1, 2]. Despite its poor prognosis, there is currently no cure for ALS, with treatments aimed at managing the symptoms to enhance the well-being of patients. Diagnostic delays of up to a year post-onset often impede early intervention efforts [1], highlighting the urgent need for tools to enable early diagnosis and prognosis evaluation to tailor treatment strategies [3].

We are aiming to employ machine learning techniques to develop a multimodal prediction pipeline to analyze neuroimaging data, obtained via advanced magnetic resonance imaging (MRI) techniques including structural, functional, diffusion and sodium imaging with an initial dataset of 7T images acquired from 20 patients suffering from ALS and 20 matched controls. The objective is to create a comprehensive, multi-modal representation of brain networks involved in ALS by integrating information from these various data modalities, through the use of graph fusion techniques. This holds promise to help identify effective markers for the disease.

Preliminary results have demonstrated potential for such an approach to help identify and classify ALS based on disease progression. Enhancing the pipeline further by integrating various modalities can allow for leveraging synergistic effects towards increasing the overall predictive power. We plan to go beyond traditional MRI analysis methods and model architectures by exploring disease biomarkers represented as graph data structures. The use of graph neural networks (GNNs) to analyze MRI data holds promise to uncover signatures and associations by taking into account the inherent structural relationships within the brain.

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Keywords: Multimodal Analysis, Biomarkers, Graph Neural Networks

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P8: Investigating a putative neurodevelopmental origin of Amyotrophic Lateral Sclerosis

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With an averaged onset between the 6th and 8th decade of life, amyotrophic lateral sclerosis (ALS) typically meets the criteria of the vast majority of neurodegenerative diseases (NDD) that manifest during late adulthood. However, growing bodies of evidence, from juvenile cases to the lethal or neurodevelopmental defects reported in mouse line that are knockout or knockdown for ALS-related genes, have contributed to the emergence of the hypothesis of a putative neurodevelopmental origin of NDD cases, including ALS. Indeed, hyperexcitability at early postnatal ages, followed by homeostatic normalization during a long period of time prior to reappearance of hyperexcitability concomitantly with disease onset was demonstrated in the motor cortex of *SOD1^{G93A}* model of ALS [1]. In the same mouse line, late embryonic and early postnatal impairment of the GABA/glycine signaling was reported in lumbar spinal motoneurons of these same animals [2]. Finally, serendipitously obtained preliminary data from my host lab indicate that a short treatment with ivermectin (IVM), when administered during perinatal development, could completely abolish disease onset and progression, along with neurodegeneration in the *Sod1^{G86R}* mouse model of ALS. Such an effect was not reported when treating adult *SOD1^{G93A}* mice [3]. My working hypothesis is that at least a subset of ALS cases arises from infra-clinical neurodevelopmental impairment. My PhD is intended to 1) identify the optimal developmental window during which administration of IVM is efficient to abolish disease onset in the *Sod1^{G86R}* mouse model of ALS and 2) identify the cellular (electrophysiological) and molecular (transcriptomic and proteomic) mechanisms that are altered in the motor cortex and spinal cord during this window of time, along with the effects of IVM on these mechanisms.

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Keywords: Neurodevelopment; omics; electrophysiology

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P9: Paraspeckle alterations in the Amyotrophic Lateral Sclerosis muscle

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Among the proteins mutated in ALS, many like FUS and TDP43 are associated to membraneless subnuclear bodies called paraspeckles. They are formed by the long non-coding RNA NEAT1_2 and RNA-binding proteins and act as molecular sponges [1]. Paraspeckles are upregulated in ALS motorneurons [2], but their status in ALS muscles is unknown. As a lot of alterations occur in ALS muscles including RNA processing and mitochondrial homeostasis, pathways regulated by paraspeckles [3], we wonder whether paraspeckles could be involved in the degeneration of the motor unit especially at the level of muscle.

To answer to this question, we are characterizing the status of paraspeckles in ALS muscles, by analyzing the expression of NEAT1_2, the number and size of paraspeckles. We showed an alteration of paraspeckles both *in vivo* and *in vitro* with an overexpression of NEAT1_2 in ALS mouse and human muscles, associated with an increase of paraspeckle number (in mouse). In primary cell cultures obtained from human deltoid muscle biopsies, we observed that NEAT1_2 is less soluble in sALS myotubes compared to the controls, indicating a paraspeckle increase. However, at this stage, we observed significantly less paraspeckles per nucleus in ALS myotubes compared to controls. These apparent contradictions suggest an alteration in the shape or size of the paraspeckles in ALS myotubes. In addition, preliminary results suggest that in ALS muscle cells, paraspeckles do not respond to cellular stress as in controls. Our results suggest paraspeckle dysfunctions in ALS muscle cells that need further investigating. We are studying the effects of paraspeckles modulation on different cellular processes that are altered in ALS (myogenesis, mitochondrial homeostasis) to shed light on the role of these bodies and better understand the physiopathology of ALS. Paraspeckles could potentially appear as relevant therapeutic targets or biomarker of the fatal neurodegenerative disease.

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Keywords : ALS, Muscle, Paraspeckles

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P10: One-carbon metabolism in corticospinal neuron development of mouse models of Amyotrophic Lateral Sclerosis

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Like the majority of neurodegenerative diseases, ALS mostly manifests during adulthood, implicitly suggesting that it hits the fully mature central nervous system. However, the evidence of a long prodromal phase, the existence of juvenile forms of ALS, and the developmental roles of genes implicated in familial cases suggest that the disease may take its root during development [1]. Interestingly, neurodevelopmental and neurodegenerative diseases share mechanisms including alterations in one-carbon metabolism (1Cmet). 1Cmet intertwines the folate and methionine cycles, is controlled by four key enzymes (DHFR, MTHFR, MAT2A and AHCY), and is central to purine synthesis and cellular proliferation, and to methylation and epigenetic regulation [2]. My PhD project aims at investigating whether ALS might arise from a neurodevelopmental impairment of 1Cmet, focusing on the motor cortex and corticospinal neurons (CSN, upper motor neurons) using transcriptomics, metabolomics and epigenetics in the *Sod1*^{G86R} and *Fus*$^{+/\Delta NLS}$ mouse models of ALS. The first results indicate that *Dhfr* expression is significantly increased in cortical progenitors (CPs) of both ALS mouse models at embryonic day E13.5, when they are generating CSN, but not at later time points. This is associated with an increased proliferation of the CPs revealed by BrdU labeling. Work is ongoing to fully characterize 1Cmet alterations in *Sod1*^{G86R} and *Fus*$^{+/\Delta NLS}$ embryos by dosing 1Cmet metabolites by liquid chromatography-mass spectrometry, and the extent of those changes on the epigenetic landscape of CPs. Because 1Cmet strongly relies on diet, this project may not only inform on the consequences of 1Cmet alterations in familial cases but also in sporadic ALS cases.

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Key words: neurodevelopment, 1C metabolism, epigenetics

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P11: *CHCHD10*$^{S59L/+}$ mouse model: behavioral and neuropathological features of frontotemporal dementia

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CHCHD10-related disease causes a spectrum of clinical presentations including mitochondrial myopathy, cardiomyopathy, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We generated a knock-in mouse model bearing the p.Ser59Leu (S59L) *CHCHD10* variant. *Chchd10*$^{S59L/+}$ mice have been shown to phenotypically replicate the disorders observed in patients: myopathy with mtDNA instability, cardiomyopathy and typical ALS features (protein aggregation, neuromuscular junction degeneration and spinal motor neuron loss) [1]. Here, we conducted a comprehensive behavioral, electrophysiological and neuropathological assessment of *Chchd10*$^{S59L/+}$ mice. These animals show impaired learning and memory capacities with reduced long-term potentiation (LTP) measured at the Perforant Pathway-Dentate Gyrus (PP-DG) synapses. In the hippocampus of *Chchd10*$^{S59L/+}$ mice, neuropathological studies show the involvement of protein aggregates, activation of the integrated stress response (ISR) and neuroinflammation in the degenerative process. These findings contribute to decipher mechanisms associated with *CHCHD10* variants linking mitochondrial dysfunction and neuronal death. They also validate the *Chchd10*$^{S59L/+}$ mice as a relevant model for FTD, which can be used for preclinical studies to test new therapeutic strategies for this devastating disease [2].

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Keywords : *CHCHD10*, DFT, ALS

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P12: Analysis of the KIF5A gene in a population of patients with ALS

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The *KIF5A* gene encodes the Kinesin heavy chain Isoform 5A (KIF5A), a protein responsible for anterograde axonal transport in neurons. Mutations in this gene are associated with a wide range of motor neuron diseases, including hereditary spastic paraplegia (SPG10), Charcot-Marie-Tooth disease (CMT), and amyotrophic lateral sclerosis (ALS). In ALS, variants primarily affect the globular domain of

KIF5A and disrupt the splicing of exon 27, which could result in the addition of a specific aberrant amino acid sequence at the C-terminal end of the protein. Early studies suggested that mutations in *KIF5A* lead to a loss of function, mainly due to exon 27 skipping in the mutated transcript. However, more recent studies indicate that the mutated forms of KIF5A, abnormally activated, may exert a dominant negative toxic effect in cellular and animal models.
We analyzed this gene in a large cohort of French patients with ALS (550 patients) and identified, through whole exome data analysis, 8 rare heterozygous variants, including a nucleotide deletion in exon 27 leading to a frameshift, and 2 intronic variants affecting intron 26. Within a family, we confirmed that one of these intronic variants segregated in a father and his daughter. Analysis of lymphoblasts from these two individuals showed that this intronic variant led to the creation of an acceptor site, causing a frameshift in the mRNA, which was then processed by the nonsense-mediated decay, supporting the loss-of-function hypothesis. Conversely, mRNA analysis of fibroblasts from the patient carrying the deletion showed that it was not degraded, suggesting a dominant negative effect. To complete these results, RT-qPCR experiments and protein level analyses are ongoing on these patient cells. Since the study of lymphoblasts may present limitations, neuropathological analysis is granted in order to examine the expression of truncated forms in patients' neurons.
Keywords: Amyotrophic Lateral Sclerosis, genetics, KIF5A
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P13: Flunarizine influences transcriptome expression in spinal muscular atrophy

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Infantile spinal muscular atrophy (SMA) is characterized by a progressive loss of motor neurons and muscle atrophy. SMA disease is caused by mutations or deletions of the *Survival Motor Neuron 1* (*SMN1*) gene leading to a deficiency of SMN protein. *SMN2* gene, homologous gene of *SMN1*, partially compensates for *SMN1* alterations by producing low levels of SMN protein [1]. SMN complex is involved in various aspects of RNA metabolism and therefore its deficiency perturbs many transcripts including non-coding RNAs. Three innovative therapies increase SMN protein levels but improve patient phenotype to variable degrees [2]. A better understanding of disease mechanisms will help to develop adjuvants therapies for low responder patients. We have previously shown flunarizine (Fz) to improve disease phenotype in the Taiwanese SMA mouse model, but its mode of action remains elusive. Fz is known as a non-specific calcium channel blocker and identified as splicing modulator. To identify Fz-modulated cellular pathways, we have used a combination of mRNA- and micro-RNA (miR)-sequencing approaches with spinal cords of Fz-treated SMN-deficient mice. There are ≈600 Fz-regulated genes (FC ≥1.3, p-value ≤0.05). Pathway analyses reveal transcriptomic changes pointing to microglial cells. Moreover, we identify potential interactions between Fz-regulated miRs and mRNAs that are under validations by RT-qPCR in spinal cords of Fz-treated mouse models. Finally, focusing on

genes critical for motor neuron function, we use murine motor neuron-like NSC34 cells to perform miR-mimic or miR inhibitor transfections for miR-128 and RT-qPCR with immunodetection studies for our validation experiments. These data further indicate the role of RNA metabolism in the SMA pathogenesis and will allow us to identify miR-mRNA network involved in motor neuron disease. Our study will uncover Fz-modulated pathway(s) as potential target(s) to improve SMA phenotype and identify new biomarkers in motor neuron diseases that are unmeet medical needs.

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Keywords

Spinal Muscular Atrophy, flunarizine, RNA.

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P14: Practices and Representations of Food of patients with Amyotrophic Lateral Sclerosis

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Nutritional management for people with ALS receives particular attention, as evidenced by the recommendations and advice provided by the Centers [1].

To propose a different perspective on the disease and give a voice to those affected, we questioned the evolution of social representations [2] of food during the course of the disease, as well as the strategies implemented by those affected by the pathology to cope with disabilities. In other words, we aimed to understand whether the onset of the disease altered eating habits, the foods that should be consumed, or dietary and aesthetic norms.

With this in mind, we conducted and analyzed 19 semi-structured interviews (totaling over 30 hours of interviews and 100 hours of transcription) with people affected by the disease. Our communication proposal aims to report on the experience conducted, as well as the results obtained. Among these, we were able to show that:

- The ways of understanding food and what is considered "good" to eat do not evolve with the onset of the disease and the provided advice. Eating habits remain relatively stable.
- Aesthetic norms remain predominant (despite the advice given to avoid weight loss), as half of the interviewed individuals continue to "watch" their weight despite the disease. In this context, normative and dietary injunctions to avoid foods considered caloric, fatty, or sugary are still present despite the pathology.

- Social conviviality and the entourage appear as social regulators that contribute to food intake. In other words, for our interviewees, it is the act of sharing meals that increases caloric intake more than nutritional advice.
- The sick individuals and their relatives implement numerous strategies to continue "eating well" and "normally": using foods that can be consumed without utensils, choosing more accessible supermarkets, selecting dishes at restaurants, involvement of caregivers and children...

Based on the experiences and lived realities, we propose work done with and for the people affected by the disease.

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Keywords : nutrition, sociology, food decision (Nutrition, sociologie, décision alimentaire)

Conflict of interest: Nothing

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P15: Characterization of two A315T transgenic mouse models for TDP-43 proteinopathies in Amyotrophic Lateral Sclerosis

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BACKGROUND: One of the most promising therapeutic targets for Amyotrophic lateral sclerosis (ALS) is linked to TDP-43 proteinopathies. Mouse models have been developed to assist in the understanding of ALS, including models hemizygous for the Prp-TDP-43^{A315T} transgene. Yet, there is limited information available concerning the popular Prp-TARDBP^{A315T} model designed by Jackson (Jax) Labs, USA; and the novel hTDP43wtxA315T model developed by GemPharmatech Labs, Japan.

AIM: Perform a longitudinal characterization study of these two TDP-43 transgenic models and determine which can be considered a more appropriate model for ALS. This was established by providing quantitative readouts of disease progression, determine changes in protein markers in organs/tissue, and determine differences in metabolomic evolution of disease in transgenic (Tg) mice.

METHODOLOGY: We assessed phenotypic and motor changes through body weight, grip strength, gait impairment, and tail position. Tissue samples were collected for western blot analysis to inspect TDP-43 markers. Serum and dried blood spots were used for omics studies to examine differences in metabolites and lipids.

RESULTS: Throughout disease progression both Jax and GPT Tg mice showed diminished weight gain, accompanied by a reduction in motor skills. TDP-43 expression was more prominent in Jax mice, compared to GPT, as we observed a positive signal and presence of fragments in crude and insoluble protein fractions. Suggesting the TDP-43 transgene is better expressed in the Jax model. Metabolomic data demonstrated OPLS-DA score plots with two distinct clusters between WT and Tg groups in the Jax model, corresponding to specific metabolomic profiles for each group. Analysis performed on extracted metabolites from the brain of the Jax mice model demonstrated a significant difference between levels of taurine, alpha-d-glucose and d-xylose, suggesting them to be discriminatory molecules in the brain of ALS mice. Similar results are expected for the metabolomic data currently being collected from the GPT model.

KEYWORDS: Amyotrophic lateral sclerosis, TDP-43 proteinopathies, Mouse models

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CONFLICT OF INTEREST: None

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P16: Assessing the fate of the cortico-reticulo-spinal pathway in ALS

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While ALS is clinically and histopathologically defined as the combined degeneration of cortical upper motoneurons and spinal and brainstem lower motor neurons, the fate and contribution of other components of the motor system have not yet been fully elucidated. This is particularly true for the reticular formation (RF), a diffuse network of nuclei that covers an expansive portion of the brainstem, receives abundant inputs from the cerebral cortex and sends abundant outputs to the spinal cord, including direct connections onto motoneurons. Imaging studies in patients with ALS and mouse models have revealed brainstem or RF impairments [1,2]. In line with this, preliminary data from my host lab demonstrated massive protein aggregates in the brainstem and RF of end-stage *SOD1^{G37R}* mice, and reactive microgliosis and astrogliosis accompanied by the loss of large neurons in the RF of symptomatic *Sod1^{G86R}* animals. In the *SOD1^{G93A}* mouse model, spinal and brainstem neurons of V2a identity (including a subpopulation of reticulospinal neurons) were shown to transiently take over most of the inspiratory effort upon loss of phrenic motoneurons, and to degenerate later than the spinal motoneurons [3]. In this context, my PhD project aims at investigating the fate and contribution of the RF, along with its upstream cortical inputs. My working hypothesis is that the cortico-reticulospinal pathway may compensate, at least transiently, for the degeneration of upper and lower motor neurons. Using anterograde and retrograde conventional and trans-synaptic tracing tools, together with last-generation transcriptomics in mice, I intend to decipher the fate and contribution of the RF to ALS using mouse models of the disease (*Sod1^{G86R}, Fus$^{\Delta NLS}$,* and *$(G_4C_2)_{149}$C9ORF72*), as well as data and tissues from patients with ALS.

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Key words: cortico-reticulo-spinal pathway, tracing, sequencing

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P17: Exploring the link between RNA-binding of TDP-43 and its aggregation

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TDP-43 is a nuclear RNA-binding protein found in cytoplasmic inclusions of neurons in two major neurodegenerative diseases, ALS and FTLD [1]. So far, there is no efficient drug for curing these diseases. Recently, we demonstrated that tandem RNA recognition motifs (RRM) of TDP-43 bind to its RNA target in a cooperative manner through intermolecular interactions [2]. This cooperative binding of TDP- 43 to mRNA is critical to maintain the solubility of TDP- 43 in the nucleus and the miscibility of TDP- 43 in cytoplasmic stress granules [2]. More recently, we successfully developed an automated detection pipeline to assess TDP-43 self-assembly combined to functional screen in living cells. We showed that the post-translational modifications affect nuclear TDP-43 localization and consequently its functions. Currently, we explore the structural effect of a pathological mutation on residues belonging to RRM domains. This fundamental knowledge will pave the way to elucidate the mechanism of TDP-43 proteinopathy and to design small molecules interfering with TDP-43 aggregation.

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RNA-binding protein, TDP-43, RNA

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P18: A JPND initiative to assess whether long covid may increase the risk of neurodegeneration, including ALS and parkinson's disease, via glycosphingolipids

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COVID-19 has multiple symptoms and age-related severity, with a significant proportion of infections leaving debilitating symptoms (long-COVID, Post-Acute Sequelae of SARS-CoV-2 infection, PASC). Multiple papers have described neurological or psychiatric involvement with changes in lipid metabolism and as many as 65 million individuals may have long COVID worldwide. Glucosylceramide synthase/glucosylceramidases are critical nodes for envelope virus infection, ALS and Parkinson's disease, with changes in glycosphingolipids (GSLs), so the European Union has been worried whether long COVID could increase the risk of neurodegeneration, setting up a JPND grant, which we coordinated with >20 experts, including the RECOVER 2bn$ action on long COVID. There are significant mechanistic overlaps between long COVID from SARS-CoV-1 and -2 and molecular aspects in ALS and Parkinson' disease. Complex glycosphingolipids (GSLs) such as GM1, a neurotrophic 5-sugar GSL, critical for neuromuscular junctions (NMJs) is a target for SARS-CoV-2 and other envelope viruses. Humans evolved a specific form of GM1 ~2.5 million years ago. Metabolomic studies had shown that GBA2 activity was specifically increased in spinal cord of presymptomatic $SOD1^{G86R}$ mice [1], increasing ceramide and depleting glucosylceramide and more complex glycosphingolipids (GSLs) such as GM1, in neuromuscular junctions (NMJs). Glucosylceramide synthase inhibitors are deleterious in ALS models, while GBA2 inhibitors are beneficial [2] and lysosomal GBA1 mutations are the main cause of Parkinson's disease. However, while the initiative has shown several common molecular pathways between SARS-CoV-2 infection, long COVID and neurodegeneration, at present there is no evidence for an increase in incidence of neurodegenerative disorders, other than that to be expected following poorer healthcare during lockdown.

1. Bouscary A, et al. Ambroxol Hydrochloride Improves Motor Functions and Extends Survival in a Mouse Model of Familial Amyotrophic Lateral Sclerosis. Front Pharmacol. 2019;10:883.

2. Henriques A, et al. Inhibition of β-Glucocerebrosidase Activity Preserves Motor Unit Integrity in a Mouse Model of Amyotrophic Lateral Sclerosis [Internet]. Vol. 7, Scientific reports. Sci Rep; 2017 [cited 2020 Oct 8]. Available from: https://pubmed.ncbi.nlm.nih.gov/28701774/

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P19: ACT4ALS-MND Alliance on Clinical Trials for ALS-MND

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ACT4ALS-MND is a unique French clinical investigation network created in 2020 by the national rare diseases health network FilSLAN. Bringing together 22 ALS/MND expert centers with excellent national coverage, the network pools the clinical expertise of its members and a large active patient file to

develop national multicenter clinical trials (CTs) and collaborate at the European and international levels. It is hosted by the Paris Brain Institute where its operational coordination is performed. In 2022, ACT4ALS-MND was labeled by the "French Clinical Research Infrastructure Network" (F-CRIN), a national infrastructure for clinical research supported by the Ministry of Health to boost the performance of French clinical research, both industrial and academic, and its attractiveness at international level.

ACT4ALS-MND aims to develop highly relevant clinical and translational research programs, with 4 thematic pillars to address the challenges of ALS therapeutic research: develop new tools for patient stratification and precision medicine, develop innovative treatment strategies and drug repurposing, improve scientific knowledge on the disease and develop innovative CT designs and facilitate CT set-up and conduct in France. It has built close links with the European clinical research consortium TRICALS (Treatment and Research Initiative to Cure ALS) and the German network for motoneuron diseases (German MND-Net).

The network has the overall goal to increase the number of CTs in the field of ALS and MNDs in France, and the number of French patients included, with operational support for all steps of a research project: scientific expertise and methodological support for trial design, regulatory expertise, feasibility study and site selection, budget evaluation, recruitment follow-up. It aims to facilitate dialogue between academic research and industry, removing barriers to accelerate innovations for ALS and MND patients.

Since 2020, ACT4ALS-MND has been involved in 3 academic-funded projects and 10 industrially sponsored trials.

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JR10 satisfaction questionnaire

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