ENCALS

European Network to Cure ALS

ENCALS meeting 2023

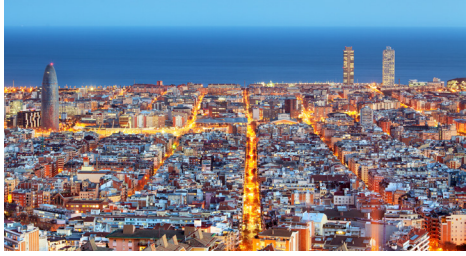
Barcelona, Spain • 11th–14th July

Oral Abstracts

https://www.encals.eu/meetings/barcelona/

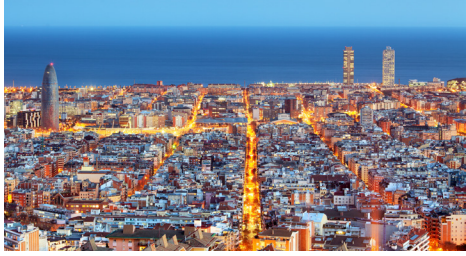
ENCALS

European Network to Cure ALS



Oral abstracts ENCALS meeting 2023

Session 1. Molecular mechanisms (Part I)



1. The role of SPP1 and perivascular fibroblasts in ALS neurodegeneration

Jianing Lin*(1), Julia Rädler(2), Samir EL Andaloussi(2), Sebastian A Lewandowski(1)

(1) Department of Clinical Neuroscience, Karolinska Institutet, Centre for Molecular Medicine, Karolinska Hospital, Stockholm, Sweden.

(2) Biomolecular Medicine, Clinical Research Center, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.

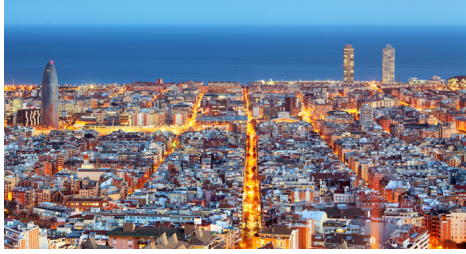
Objectives: ALS is a disease with unexplained origins and highly unpredictable life expectancy outcomes. The dynamics of disease progression are typically explained by measurements of neuron-derived inputs such as neurofilaments. However, our group has recently shown that vascular-derived mechanisms in the brain perivascular fibroblast cells can give even more accurate indication of patient survival, which points to new promising treatment modalities. Since the increase of the fibroblast-derived protein SPP1 was associated with shorter life expectancy in ALS patients, we have developed preclinical treatment modalities targeting its expression.

Methods: In this study, we designed siRNAs to target SPP1 expression, and applied them in NIH-3T3 fibroblast cell line to test their efficacy. In order to identify a subpopulation of ALS patients that would potentially benefit from SPP1 targeted treatment, we have measured SPP1 expression in 452 patients with various clinical manifestations.

Results: We have screened multiple siRNAs to inhibit SPP1 expression and validated them using NIH-3T3 cell lines to identify sequences that efficiently suppress its mRNA expression. Subsequently, we measured the SPP1 in plasma from patients with varying age at onset and life expectancies, and we identified a specific subset of ALS patients who would potentially benefit the most from the siRNA treatment.

Discussion: Recent progress in treating neuromuscular diseases has highlighted the safety and the potential of antisense oligonucleotide treatments when applied to selected candidates. The stratification of patients became a crucial factor in the success of these therapies. Our project brings a promise of a targeted therapy towards the vascular inflammatory component in carefully stratified subgroups of ALS patients.

Key words: ALS, SPP1, siRNA, treatment



2. TDP-43 loss induces cryptic 3'end processing in neurons and ALS brains

Sam Bryce-Smith* (1), Anna-Leigh Brown (1), Nicol Birsa (1), Sarah Hill (3), Matthew Keuss (1), Francesca Mattedi (1), Puja Mehta (1), Daniel Ramos (3), Sahba Seddighi (3), Kai Sun (1), Jobert Vargas (1), Oscar Wilkins (1,4), Matthew Yome (1), Matteo Zanovello (1), Michael Ward (3), Maria Secrier (2), Pietro Fratta (1)

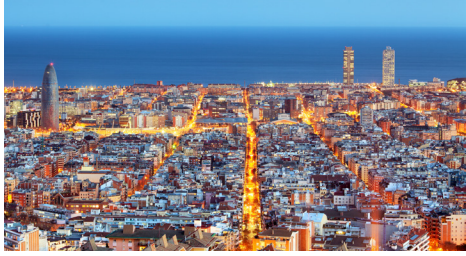
1 - UCL Queen Square Motor Neuron Disease Centre, Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UCL, London, UK

2 - Department of Genetics, Evolution and Environment, UCL Genetics Institute, University College London, London, UK

3 - National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA

4- The Francis Crick Institute, London, UK

A hallmark of sporadic ALS is nuclear depletion and cytoplasmic aggregation of the RNA-binding protein TDP-43 which occurs in an estimated 97% of cases. TDP-43 has a well-characterised nuclear function in RNA processing. One of its best studied RNA processing functions with relevance to ALS pathogenesis is repression of cryptic splicing events, of which inclusion typically leads to loss of gene expression through activation of nonsense-mediated decay (NMD). Cryptic polyadenylation (poly(A)) events are expected to be insensitive to NMD, and as such could potentiate gain-of-function effects through production of truncated proteins and/or alterations to 3'UTR regulatory content. Although TDP-43 dependent cryptic splicing has been well catalogued, cryptic polyadenylation has not been systematically explored outside of events associated with a novel 5'ss which can be identified through conventional splicing analyses. To identify novel poly(A) sites and define full last exon structures from bulk RNA-seq data, we assembled a novel computational pipeline which combines StringTie to define last exon frames and the PolyASite database to refine predicted 3' ends. We applied this pipeline to a compendium of published and in-house TDP-43 depletion datasets and identified widespread cryptic polyadenylation which could be recapitulated in neurons derived from patient brains. In a bulk RNA-seq cohort of neuronal tissue from control, ALS & FTLD patients (446 individuals; 1682 tissue samples), we focused on a cryptic 3'UTR extension in ELK1 which was selectively upregulated in tissues affected by TDP-43 proteinopathy. Using FISH in i3 cortical neurons with TDP-43 knockdown, we found the ELK1 cryptic 3'UTR is mislocalised to the cytoplasm, coinciding with a two-fold increase in ELK1 translation by Ribo-seq. To explore whether ELK1's transcription factor activity is altered in the context of TDP-43 depletion, we used published ChIP-seq data and found ELK1's target genes are consistently downregulated in RNA-seq of TDP-43 knockout HeLa cells. We further plan to investigate how the transcription factor activity of ELK1 is altered in the context of TDP-43 depletion and BDNF signalling. To conclude, we provide evidence that derepression of cryptic polyadenylation events can induce gain-of-function effects, highlighting a new layer of cryptic RNA biology in the context of TDP-43 ALS.

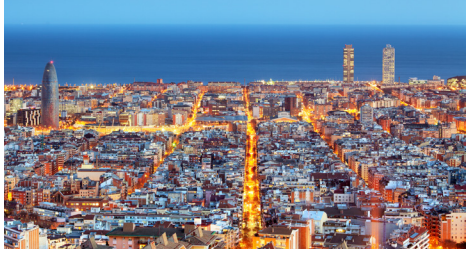


3. Involvement of oligodendrocytes in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) linked to Fused in Sarcoma protein (FUS)

Marguerite Jamet* (1), Jose-Luis Gonzalez de Aguilar (1), Luc Dupuis (1)

University of Strasbourg, INSERM, Central and peripheral mechanisms of neurodegeneration, UMR-S 1118, Biomedical research centre, Strasbourg, France.

The most severe forms of Amyotrophic lateral Sclerosis (ALS) are associated with heterozygous truncation of the NLS signal in the FUS protein, a DNA/RNA binding protein involved in gene expression regulation. This truncation results in cytoplasmic mislocalisation of FUS protein. This mislocalisation has also been observed in several cases of sporadic Fronto Temporal Dementia (FTD) called FUS-FTD. Oligodendrocytes and myelin are known to be impaired in ALS, yet the oligodendrocyte involvement in FUS-ALS and FUS-FTD remains to be explored. My PhD aims to understanding the contribution of oligodendrocytes in the onset, propagation and pathophysiology of FUS- ALS and FUS- FTD. In order to explore oligodendrocytes' contribution to ALS and FTD phenotypes, we first characterized oligodendrocyte and myelin in knock in Fus mice, a mouse model of FUS-ALS/FTD. Interestingly, Fus mice exhibited an increase of the myelin sheath thickness in the cortex and corpus callosum, associated with an increase of myelin basic protein (MBP) protein level without increased mRNA levels. Indeed, reporter assays showed that FUS was critical in MBP mRNA translation. Rescuing mutant FUS expression in mature oligodendrocytes of Fus mice was sufficient to delay ALS-like motor defects in this model and restored oligodendroglial but also neuronal protein levels, suggesting that oligodendrocyte FUS mutation acted non cell autonomously on neurons. In a complementary strategy, we created a mouse model expressing FUS truncation in oligodendrocytes only. These mice display FTD-like social abnormalities in males but not in females, suggesting a role of oligodendrocytes in cognitive impairments associated with FUS mutation in a sex-dependent manner. Collectively, our results show that FUS mutation modifies cell autonomously myelin composition and structure in oligodendrocytes, which contributes to both motor and cognitive deficits in ALS-FUS and FTD-FUS.



4. Hexanucleotide repeat expansions in C9orf72 alter microglial responses and prevent a coordinated glial reaction in ALS

Pegah Masrori (1,2,3#), Baukje Bijnens (4,5#), Kristofer Davie (6), Suresh Kumar Poovathingal (7), Annet Storm (2), Nicole Hersmus (2), Laura Fumagalli (4,5), Ludo Van Den Bosch (1,2), Mark Fiers (8), Dietmar Rudolf Thal (9,10), Renzo Mancuso(4,5,#), Philip Van Damme (1,2,3,#)*

1. KU Leuven - University of Leuven, Department of Neurosciences, Leuven Brain institute (LBI), Leuven, Belgium.
2. Laboratory of Neurobiology, VIB Center for Brain & Disease Research, Leuven, Belgium
3. University Hospitals Leuven, Department of Neurology, Leuven, Belgium.
4. Microglia and Inflammation in Neurological Disorders (MIND) Lab, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium.
5. Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.
6. Single Cell Bioinformatics Expertise Unit (CBD), VIB Center for Brain & Disease Research, Leuven, Belgium.
7. Single Cell Analytics & Microfluidics Core, VIB Center for Brain & Disease Research, Leuven, Belgium.
8. VIB Center for Brain & Disease Research, Leuven, Belgium.
9. Department of Imaging and Pathology, KU Leuven, Leuven, Belgium.
10. Department of Pathology, University Hospitals Leuven, Leuven, Belgium.
 # These authors contributed equally

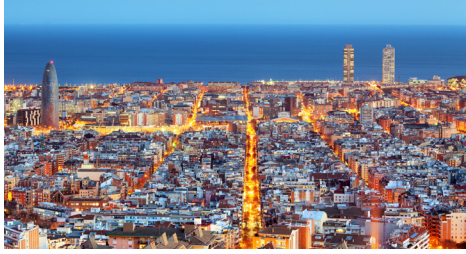
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron loss (MNs) in the motor cortex, brainstem, and spinal cord. ALS has a strong genetic component, where the most common monogenic cause is the GGGGCC hexanucleotide repeat expansions (HRE) in the chromosome 9 open reading frame 72 gene (C9orf72). Both loss-of-function (LOF) and toxic gain-of-function (GOF) mechanisms in neuronal and non-neuronal cells have been implicated in the disease pathogenesis. We performed an unparalleled single-nuclei RNA sequencing resource from paired motor cortex and spinal cord samples from sporadic ALS without any known gene mutation (sALS), ALS patients with a C9orf72 mutation (C9-ALS) and controls. We then characterized the transcriptomics profile of microglia and astrocytes.

We assessed the levels of C9orf72 and confirmed the HRE leads to reduced C9orf72 expression levels. Direct comparison between C9-ALS and sALS microglia showed that while sALS microglia display transcriptomics changes consistent with an activation state and C9-ALS microglia remained vastly homeostatic state. This results indicate that the HRE in C9orf72 impairs microglial cell state transition, consistent with a partial loss of functionality of microglial cells. Whereas sALS microglia showed increased transcription of genes involved in immune, lysosomal and phagocytic pathways, C9-ALS microglia remained similar to the controls. Interestingly, we observed that the majority of the changes occurred in the spinal cord, with only mild alterations in the motor cortex.

Similarly to what we observed in microglia, we found an overall lower extent of transcriptomic changes in C9-ALS vs. sALS astrocytes. sALS astrocytes exhibited an upregulation of genes associated to a reactive state, as opposed to C9-ALS astrocytes which more often retained a homeostatic state. This data indicates a C9orf72 HRE-mediated defective engagement in a coordinated response in ALS pathology indicative of an aberrant microglia-astrocyte interaction.

Overall we present that C9orf72 HRE results in reduced C9orf72 expression levels in the microglia and a subsequent reduction in their response to disease, consistent with deficits in phagocytic and lysosomal pathways. We hypothesise that this leads to a defective general glial response that extends to astrocytes, hindering a coordinated response that increases the risk for developing ALS.

Session 2. Molecular mechanisms (Part II)



5. NEK1 loss-of-function mutation impairs ciliogenesis in iPSC-motoneurons

Marta Nice Sorce*(1), Sabrina Invernizzi(1), Chiara Lattuada(1), Serena Santangelo(2), Valeria Casiraghi(2), Alberto Brusati(3), Alessio Silva(1), Vincenzo Silani(1)(4), Patrizia Bossolasco(1), Antonia Ratti(1)(2)

1 IRCCS Istituto Auxologico Italiano, Department of Neurology and Laboratory of Neuroscience, Milan, Italy

2 Department of Medical Biotechnology and Molecular Medicine, Università degli Studi di Milano, Milan,Italy

3 Department of Brain and Behavioral Sciences, Università degli Studi di Pavia, Pavia, Italy

4 Department of Pathophysiology and Transplantation, Università degli Studi di Milano, "Dino Ferrari Center", Milan,Italy

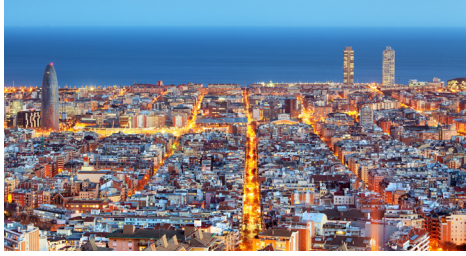
Mutations in NEK1 gene account for nearly 3% of familial and sporadic ALS cases. The survival time of heterozygous loss-of-function (LOF) mutation carriers is significantly shorter than that of missense variant carriers. NEK1 codes for a protein kinase involved in several biological pathways, including DNA damage repair, mitochondrial functionality and ciliogenesis. However, the pathomechanisms whereby NEK1 LOF leads to neurodegeneration in ALS have not been clarified so far.

Aim of our study was to investigate the biological effects of NEK1 LOF mutations using motoneurons derived from human induced pluripotent stem cells (iPSC-MN).

Primary fibroblasts from a healthy control were reprogrammed into iPSC and a NEK1 LOF mutation (p.Arg261Profs19) was introduced through CRISPR/Cas9 gene editing, leading to NEK1 protein haploinsufficiency. The DNA damage response was evaluated after exposing iPSC-MN to the radiomimetic agent Neocarzinostatin, that causes DNA double-stranded breaks. NEK1 LOF iPSC-MN showed a similar capacity to repair DNA breaks as control cells, but they exhibited abnormalities in mitochondrial structure and biogenesis. Our findings also revealed that a decreased number of NEK1 LOF iPSC-MN formed cilia, which were also significantly shorter, compared to control iPSC-MN. We also generated 3D brain organoids to evaluate whether

impairment of cilia formation and mitochondria organization is also confirmed or influenced by non-cell autonomous mechanisms.

Altogether our results support that NEK1 serves as a hub signalling kinase for mitochondrial functionality and cell fate determination via cilia organelles. The involvement of ciliogenesis dysfunction in ALS might also provide novel targets and therapeutic strategies to be further tested in iPSC-derived 2D and 3D disease models.



6. CRISPR/Cas9 screen in human iPSC-derived cortical neurons identifies NEK6 as a novel disease modifier of C9orf72 poly(PR) toxicity

Wenting Guo(1,2,3)*, Haibo Wang(4,5), Arun Kumar Tharkeshwar(2,3), Julien Couthouis(6), Elke Braems(2,3), Pegah Masrori (2,3,7), Evelien Van Schoor(2,3,8), Yannan Fan(1), Karan Ahuja(1), Matthieu Moisse(2,3), Maarten Jacquemyn(9), Rodrigo Furtado Madeiro da Costa(1), Madhavsai Gajjar(1), Sriram Balusu(3), Tine Tricot(1), Laura Fumagalli(2,3), Nicole Hersmus(2,3), Rekin's Janky(10), Francis Impens(11,12,13), Pieter Vanden Berghe(14), Ritchie Ho(15), Dietmar Rudolf Thal(8), Rik Vandenberghe(7,16), Muralidhar L. Hegde(4,5), Siddharthan Chandran(17,18), Bart De Strooper(3,17), Dirk Daelemans(9), Philip Van Damme(2,3,7), Ludo Van Den Bosch(2,3), Catherine Verfaillie(1)

(1). KU Leuven-Stem Cell Institute (SCIL), Leuven, Belgium.

(2). KU Leuven-University of Leuven, Department of Neurosciences, Experimental Neurology, and Leuven Brain Institute (LBI), Leuven, Belgium.

(3). VIB, Center for Brain & Disease Research, Leuven, Belgium.

(4). Division of DNA Repair Research, Department of Neurosurgery, Center for Neuroregeneration, Houston Methodist Research Institute, Houston, TX, United States.

(5). Department of Neuroscience Research at Neurological Surgery, Weill Medical College, New York, NY, United States.

(6). Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.

(7). University Hospitals Leuven, Department of Neurology, Leuven, Belgium.

(8). Laboratory of Neuropathology, Department of Imaging and Pathology, KU Leuven, Leuven Brain Institute (LBI), Leuven, Belgium.

(9). KU Leuven Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium.

(10). VIB Nucleomics Core, Leuven, Belgium.

(11). VIB Center for Medical Biotechnology, Ghent, Belgium.

(12). Department of Biomolecular Medicine, Ghent University, Ghent, Belgium.

(13). VIB Proteomics Core, Ghent, Belgium.

(14). KU Leuven – University of Leuven, Translational Research Centre for Gastrointestinal Disorders, Leuven, Belgium.

(15). Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA.

(16). KU Leuven-Laboratory for Cognitive Neurology, Department of Neurosciences, Leuven Brain Institute, Leuven, Belgium.

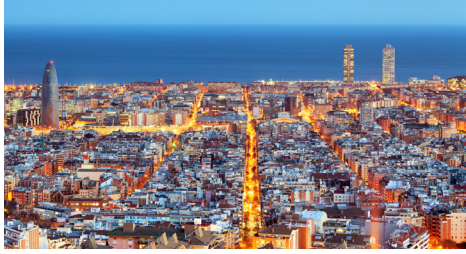
(17). UK-Dementia Research Institute at University College London, London, UK.

(18). Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK.

Introduction: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating neurological diseases, with clinical, pathologic and genetical overlaps. Hexanucleotide-repeat expansions in C9ORF72 are the most common genetic cause of ALS/FTD. One leading hypothesis underlying the pathogenesis of C9ORF72 expansion is the gain of toxic function from the dipeptide repeats proteins (DPRs) that are translated from the repeat expansions. Among DPRs, the most toxic ones being those containing arginines (e.g. poly(PR)). Therefore, finding new modifiers to reverse poly(PR) toxicity is vital important to provide effective therapeutic approaches for C9orf72 related ALS/FTD.

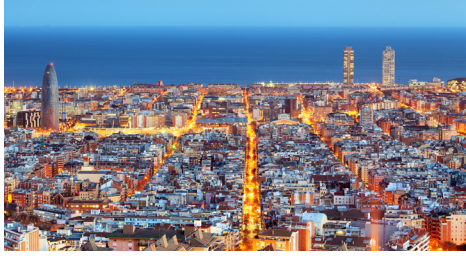
Methods: We performed a CRISPR/Cas9 knock-out screen in human Induced pluripotent stem cells (iPSC) derived neurons to identify new disease modifiers of C9orf72 poly(PR) toxicity. We validated the candidate modifiers by using in vitro, in vivo, and ex-vivo models of ALS/FTD.

Results: NIMA-related kinase 6 (NEK6) came out as one of the top hits from the CRISPR/Cas9 screen. Decreased NEK6 expression rescued human iPSC-derived cortical neuron cell death caused by high concentrations of poly (PR) and rescued axonal transport defects caused by low concentrations of poly (PR). In addition, knock-down NEK6 in neurons differentiated from C9orf72 patient-derived iPSCs also rescued axonal transport defects. Consistently, morpholi-



no-mediated knock-down of nek6 in zebrafish embryos, also significantly rescued the poly (PR)-induced motor axonopathy, validating the role of NEK6 in DPR mediated axonopathy in vivo. Furthermore, we also found that the NEK6 expression level is dysregulated in peripheral blood cells and postmortem brain tissues of C9orf72 ALS/FTD patients. At the mechanistic level, we found that knock-down and pharmacological inhibition of NEK6 rescued DNA damage and p53 pathway upregulation in neurons differentiated from C9orf72 patient-derived iPSCs.

Conclusion & Discussion: Our study discovered, validated, and explored the therapeutic potential of the kinase, NEK6, to rescue poly(PR) toxicity from C9ORF72 repeat expansion. We demonstrated a new role for NEK6 in p53 mediated DNA damage and neuronal toxicity. Our data strongly indicated that targeting NEK6 might be a promising therapeutic strategy for C9orf72 related ALS/FTD. It will be crucial to develop clinically relevant NEK6 inhibitors with minimal side effects suitable for treatment of C9orf72 patients and beyond.



7. SIRT1 upregulation mitigates DPR induced toxicity in C9orf72-associated disease models

S. Imhof(1,2), T. Akiyama(3), H. Caliskan(1,2), J. Rubin-Sigler(4), E. Metzl Raz(3), F. Walter(1,2,5), O. Keritam(1,2), C. Hotzy(1,2), F. Zimprich(1,2), E. Gelpi(1,2,7), S. Klotz(1,2,7), J. Ichida(4), V. Nagy(1,6,8), A. Gitler(3), H. Cetin(1,2)

(1)Department of Neurology, Medical University of Vienna, Vienna, Austria. (2)Comprehensive Center for Clinical Neurosciences & Mental Health, Medical University of Vienna, Vienna, Austria. (3)Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA. (4)Department of Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of Southern California, Los Angeles, CA, USA. (5)Ludwig-Maximilians-Universität Munich, Faculty of Biology, Biocenter, Munich, Germany. (6) Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria. (7)Division of Neuropathology and neurochemistry, Medical University of Vienna, Vienna, Austria. (8)CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.

BACKGROUND

SIRT1,a NAD+-dependent deacetylase, is crucial for the maintenance of heterochromatin structure, nucleolar organization and promotes DNA damage repair, all of which were shown to be compromised by dipeptide repeat proteins (DPRs). DPRs result from the translation of the GGGGCC-hexanucleotide C9orf72-repeat expansion as the most common monogenetic cause of ALS (C9-ALS) and frontotemporal dementia (C9-FTD).

AIM

The aim of our study was to investigate the role of SIRT1 in different C9orf72-associated disease models, and to test its utility as a novel therapeutic target.

METHODS

The mCherry-tagged DPRs with a length of 50 repeats were transfected into N2a mouse cells to assess DPR specific effects. Our findings were validated in induced pluripotent stem cells (iPSCs) and post-mortem brain tissue of C9-ALS/FTD patients, sporadic ALS (sALS) patients or controls.

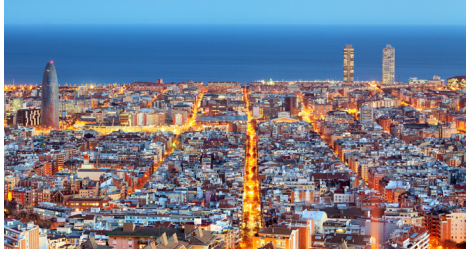
RESULTS

The transfection of N2a cells with DPRs resulted in genomic instability as revealed by i) disintegration of nucleoli ii) reduced heterochromatin formation, iii) increased DNA double strand breaks, and iv) an accumulation of p53. SIRT1 RNA and protein levels were found to be decreased upon transfection of N2a cells with poly-GR or poly-GA. These findings could be confirmed in iPSCs derived from C9-ALS patients (C9-iPSCs), in which SIRT1 levels were significantly lower when compared to isogenic controls. In line with these findings, nuclear SIRT1 expression was also significantly lower in frontal cortices of C9-ALS patients as compared with brain tissue of sALS patients and controls, and these changes were accompanied by the cytoplasmic accumulation of a 75 kDa SIRT1 isoform in C9-ALS patients.

Treatment of N2a cells with resveratrol led to increased SIRT1 expression and mitigation of the previously reported surrogates of genomic instability. In iPSCs, resveratrol treatment resulted in the same ameliorations and overall increase of cellular viability. The restorative effect of resveratrol on genomic instability was SIRT1-dependent.

CONCLUSION

Our study provides compelling evidence that the toxicity mediated by the DPRs poly-GR and poly-GA depends on the suppression of SIRT1 activity, and that the restoration of SIRT1 activity by resveratrol is sufficient to mitigate genomic instability and increase cellular viability in transfected N2a cells and/or C9-iPSCs. Rescuing SIRT1 activity may therefore represent a promising therapeutic strategy in C9orf72-associated diseases.



8. TBK1 loss-of-function is associated with cell autonomous microglial dysfunction

Uroosa Chughtai* (1,2), Raja Nirujogi (3), Daniel Cabezas De La Fuente (4), Gaynor Smith (1,4), Meng Li (2,4), Dario Alessi (3), Owen Peters (1,2)

(1) UK Dementia Research Institute, Cardiff University, Cardiff, United Kingdom.
(2) School of Biosciences, Cardiff University, Cardiff, United Kingdom.
(3) MRC Protein Phosphorylation & Ubiquitylation Unit, University of Dundee, Dundee, United Kingdom.
(4) School of Medicine, Cardiff University, Cardiff, United Kingdom.

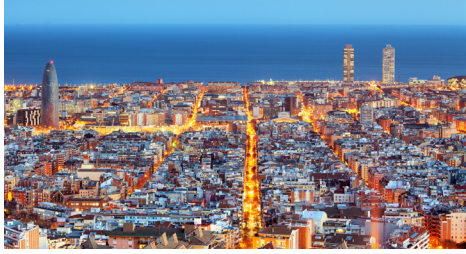
Heterozygous loss-of-function mutations in TANK-binding kinase 1 (TBK1) have been associated with sporadic and familial forms of ALS and FTD. TBK1 is a ubiquitously expressed serine/threonine protein kinase central to numerous cellular signalling pathways, including autophagy and immune signalling. Whilst cell autonomous dysfunction in neurons has been shown to contribute to ALS/FTD pathogenesis following TBK1 haploinsufficiency, the impact of TBK1 loss-of-function in microglia is less well understood. TBK1 is highly expressed in microglia, has an established role in the peripheral innate immune system, and interacts with other key ALS/FTD-associated genes, suggesting that disease-associated TBK1 mutations may cause microglial dysfunction in ALS/FTD. We aimed to investigate the molecular function of TBK1 in microglia and understand how ALS/FTD-associated TBK1 mutations may intrinsically impair microglial function to drive ALS/FTD disease pathogenesis.

We used a combination of pharmacological TBK1 kinase inhibition and genetic TBK1 deletion to model TBK1 loss-of-function in immortalised microglial cell lines and human pluripotent stem cell-derived microglia. Subsequently we investigated microglial (dys)function using unbiased TMT-based LC-MS/MS quantitative proteomics followed by targeted functional assays.

We found that TBK1 kinase inhibition results in dysregulation of the global microglial proteome, with 143 proteins becoming significantly differentially expressed. Gene ontology enrichment and protein-protein interaction analysis of up- and downregulated protein sets highlighted significant dysregulation in interferon signalling, as well as deficits in phagocytosis and antigen presentation. Live-cell timelapse confocal microscopy confirmed deficits in phagocytosis, with TBK1 kinase inhibition resulting in reduced microglial uptake of E. Coli in a dose-dependent manner. Macropinocytic uptake of dextran was also found to be deficient, whilst clathrin-mediated endocytic uptake of transferrin remained unaffected.

In conclusion, we have shown that TBK1 loss of kinase function results in microglial dysfunction in a cell autonomous manner. Future work aims to further understand the role of TBK1 in phagocytosis and related pathways, as well as identify microglial TBK1 substrates and understand how microglial TBK1 loss-of-function may be leading to neuronal dysfunction using proteomics approaches in TBK1-/- hPSC-derived microglia.

Session 3. Proteomics



9. Characterization of the human spinal cord synaptic proteome in ALS

Zsofia I Laszlo* (1), Nicole Hindley (1), Douglas J. Lamont (2), Alberto Catanese (3), Chris Henstridge (1)

(1) Division of Cellular and Systems Medicine, School of Medicine, University of Dundee, Dundee, Scotland, UK.

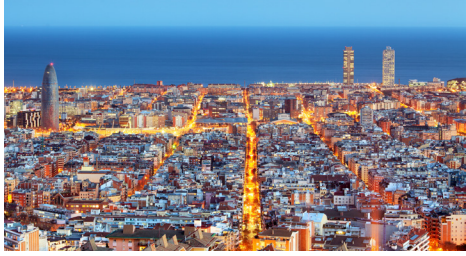
(2) FingerPrints Proteomics Facility, Discovery Centre, School of Life Sciences, University of Dundee, Dundee, Scotland, UK.

(3) Institute of Anatomy and Cell Biology, Ulm University, Ulm, Germany.

Synapse loss is an early feature of neurodegeneration in ALS, affecting both the CNS and periphery at the neuromuscular junction. Recent evidence showed that synaptic dysfunction actively contributes to disease progression and influences the behaviour of other non-neuronal cell types, such as microglia. Therefore, understanding the underlying molecular changes within the synapses and their role in cellular interactions is crucial.

To unravel the destructive molecular changes behind synapse dysfunction and loss, we isolated synaptoneurosome fractions from post-mortem human spinal cord tissue from sporadic and C9ORF72-RE-positive patients and age- and gender-matched controls and proteomics profiling was performed using tandem mass tag (TMT) labelling and liquid chromatography-mass spectrometry (LC-MS). Synaptic fractions and proteomics results were further validated using molecular biology tools. Overall, more than 6000 proteins were identified and revealed expression changes in more than 2500 proteins, giving a unique profile of the ALS synapses. Interestingly, the majority of up- and downregulated proteins were congruent between the sporadic and the familial groups showing similar synaptic alterations regardless of genetic background. The upregulated protein entries mainly showed enrichment in terms of native immunity, neuroinflammation and molecular binding, meanwhile, downregulated proteins were arranged around biological processes such as synaptic signalling, cellular respiration and synaptic vesicular release. Surprisingly, we also identified 39 ALS-related genes in the post-mortem spinal cord synapses coding pathogenic familial subtypes. Moreover, to investigate the potential molecular machinery behind synapse loss we found diverse signalling pathways connected to lysosomal dysfunction, autophagy and molecular markers for glial cell recognition and phagocytosis.

Together, these results greatly increase our understanding of ALS synaptic pathology, highlight novel molecular pathways and cellular interactions, and provide a potential platform for further target identification and drug discovery.



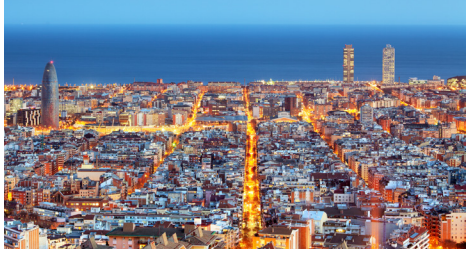
10. Characterising the cortical synaptic proteome of Amyotrophic Lateral Sclerosis (ALS).

Nicole Hindley*(1), Dr Zsofia Lazslo(1), Dr Rachel Kline(2), Dr Tom Wishart(2), Dr Christopher Henstridge(1)

(1)School of Medicine, University of Dundee, Ninewells Hospital, Dundee, Scotland, UK(.)
(2)University of Edinburgh, Edinburgh, Scotland, UK.

Synaptopathies and synaptic loss are becoming increasingly recognised as central factors in neuropathies. Synaptic dysfunction and loss are thought to be one of the earliest pathological events in many neurodegenerative diseases, including ALS. Further, increased synapse loss has been shown in the prefrontal cortex of ALS patients who present with cognitive impairment, which around 50% of ALS patients experience in addition to impaired motor function. This cognitive impairment can be so severe that ~15% of ALS patients are diagnosed with co-morbid Frontotemporal Dementia (ALS-FTD).

Despite the profound synaptic pathology in both ALS and FTD, the molecular mechanisms underlying these synaptic abnormalities are still unknown. To address this, we generated synaptoneurosome (SNS) fractions from human primary motor cortex (BA4) and dorsolateral prefrontal cortex (BA9) control (n=11) and ALS tissue (n=18). ALS samples were stratified by the presence of cognitive impairment based on the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) score and C9ORF72 hexanucleotide repeat expansion (C9ORF72-RE) (the most common genetic cause of ALS and FTD) status. Through pooling these samples and conducting TMT-LC MS/MS proteomics we identified >6000 proteins in our SNS samples. Enrichment analysis of our data showed high enrichment for synapses and synaptic processes, and we validate our approach with western blotting and array tomography. Further, we identified protein changes specific to stratifications of cognitive impairment and C9ORF72-RE status. Interestingly, KEGG analysis of protein changes specific to the C9ORF72-RE group showed strong enrichment for postsynaptic processes and glutamate receptor signalling. Utilisation of robust unbiased bioinformatic analyses identified protein expression clusters for group comparisons of interest, which were taken forward for further enrichment analysis. Subsynaptic localisation of dysregulated proteins were also identified in each comparison, revealing many postsynaptic protein changes in C9ORF72-HRE. Developing the field's understanding of the molecular processes behind this synaptic pathological phenomenon, across multiple different ALS stratified cohorts, could assist in the development of therapeutics to target ALS pathology at its earliest stage to prevent or slow down disease progression.

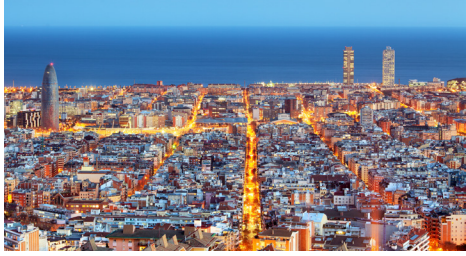


11. Multiomic profile integration reveals early disease signatures of Amyotrophic Lateral Sclerosis

Lucas Caldi Gomes*(1), Sonja Hänzelmann, Sergio Oller(2), Mojan Parvaz (2), Fabian Hausmann(2), Robin Khatri(2), Melanie Ebbing(2), Constantin Holzapfel(2), Johanna Knöferle(1), Isabell Cordts(1), Antonia Demleitner(1), Laura Tzeplaeff(1), Marcus Deschauer(1), Marc Sturm(3), Tobias Haack(3), Sebastian Streb(4), Magdalena Kuzma-Kozakiewicz(5), Marie Gebelin(6), Christine Carapito(6), Pavol Zelina(7), Marta Canizares Luna(7), R. Jeroen Pasterkamp(7), Laura Pasetto(8), Valentina Bonetto(8), Qihui Zhou(9), Dieter Edbauer(9), Endre Laczko(4), Hubert Rehrauer(4), Ralph Schlapbach(4), Stefan Bonn(2), Paul Lingor(1).

(1)Rechts der Isar Hospital, Technical University of Munich, Munich, Germany.
(2)University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
(3)University Hospital and Faculty of Medicine, Eberhard Karls University Tübingen, Tübingen, Germany.
(4)Functional Genomics Center Zurich (FGCZ), Zurich, Switzerland.
(5)Medical University of Warsaw, Warsaw, Poland.
(6)Hubert Curien Multi-disciplinary Institute (IPHC), University of Strasbourg, Strasbourg, France.
(7)University Medical Center, Utrecht Brain Center, Utrecht, The Netherlands.
(8)Mario Negri Institute for Pharmacological Research, Milano, Italy.
(9)German Center for Neurodegenerative Diseases (DZNE), Munich, Germany.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder with a long diagnostic delay and poor prognosis. Therapeutic options are insufficient and a better characterization of early pathological changes in ALS is urgent. Here we profiled early molecular changes in ALS brains using multiomics analyses. Human postmortem prefrontal cortex (PFC) tissue of sporadic ALS patients (n=51) and controls (n=50) as well as PFC tissue from 4 transgenic mouse models (mutations for SOD1;TDP43;C9orf72;FUS) were analyzed by multiple deep omics methods, including transcriptomics, miRNAomics (Illumina NovaSeq 6000/HiSeq 2500), and proteomics (label-free LC-MS (nanoLC-MS/MS)). Differential expression analyses were performed with custom frameworks in R, including an unbiased Multiomics Factor Analysis. Functional annotation of differentially abundant RNA and proteins revealed enrichment for multiple disease-relevant mechanisms, such as cell survival, extracellular matrix, oxidative stress/mitochondrial function, lipid metabolism, GTP metabolism, RNA processing, synaptic function and immune response. Multifactorial data integration for the human data underlined mechanisms that were initially identified in the individual omics, but also highlighted further important molecular hubs (TDP-43 pathology/MAPK pathway). Both individual and integrated multiomic results were characterized by marked sex-specific differences, which were more pronounced in males than in females. Altered pathways differed not only in the extent of their deregulation, but both sexes also showed different deregulated pathways. Furthermore, our data show that there was a marked heterogeneity on the molecular level for the analyzed samples which cannot be explained by correlation to clinical features. In the mouse models, C9orf72 animals displayed the most pronounced alterations at all levels, corresponding to its severe clinical phenotype. TDP43, SOD1 and FUS models showed less pronounced changes, and also less severe phenotypes. Disease-associated pathways enriched for the transgenic mouse models only partially overlap with human results, indicating that the models reflect only subsets of the pathology of sporadic ALS in humans. In summary, our multiomic profiling approaches revealed strong sex-specificity of ALS-related disease alterations, a remarkable molecular heterogeneity as well as novel disease-relevant pathways, which could represent viable therapeutic targets for ALS.



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

Laura Rodríguez-Gómez* (1), Oihane Pikatza-Menoio (1, 2), Maddi Garciandia-Arcelus (1), Andrés Jiménez-Zúñiga (1), Jon Ondaro-Ezkurra (1, 2), Gorka Guereñu-Lopetegi (1, 2), Adolfo López de Munain (1, 2, 3, 4), Sonia Alonso-Martín (1,2), Francisco Javier Gil-Bea (1, 2, 5)

(1) Neuroscience Area, Biodonostia Research Institute, San Sebastián, Spain.

(2) CIBERNED (Network Center for Biomedical Research in Neurodegenerative Diseases), Carlos III Institute, Madrid, Spain.

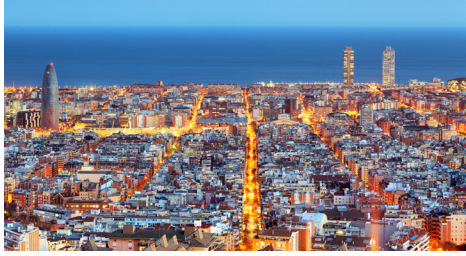
(3) Neurology Department, Donostia University Hospital, OSAKIDETZA, San Sebastián, Spain.

(4) Neurosciences Department, Basque Country University, San Sebastián, Spain.

(5) Ikerbasque Basque Foundation for Science, Bilbao, Spain.

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

Session 4. Genomics and Transcriptomics



13. A Single-nuclei RNA Sequencing Approach of Oligodendrocyte Dysfunction in ALS.

Maria Georgopoulou * (1,2,4,5), Pegah Masrori (1,4,5,6), Dietmar Thal (3,7), Alejandro Sifrim (2,4), Philip Van Damme (1,4,5,6)

1. Laboratory for Neurobiology, Department of Neurosciences, KU Leuven (University of Leuven), Leuven Brain Institute (LBI), Leuven, Belgium.

2. Laboratory of Multi-omic Integrative Bioinformatics (LMIB), Department of Human Genetics, Ku Leuven (University of Leuven).

3. Laboratory for Neuropathology, Department of Imaging and Pathology, Leuven Brain Institute (LBI) KU Leuven, Leuven, Belgium.

4. KU Leuven - University of Leuven, Department of Neurosciences, Leuven Brain institute (LBI), Leuven, Belgium.

5. Center for Brain and Disease Research, VIB, Leuven, Belgium.

6. University Hospitals Leuven, Department of Neurology, Leuven, Belgium.

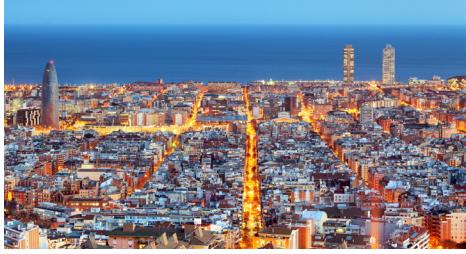
7. University Hospitals Leuven, Department of Pathology, Leuven, Belgium.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder primarily affecting the motor system, which has a strong genetic component. The most common gene mutation causing ALS is a hexanucleotide repeat expansion in C9orf72. The mechanisms leading to motor neuron degeneration are not fully understood. It has become clear that ALS is a non-cell autonomous disease in which glial cell types are important as well.

We used Single-nuclei RNA sequencing to examine the differences in gene expression in post-mortem brain(15) and spinal cord(15) tissues of individuals with sporadic ALS(5), C9orf72-ALS(5), and control individuals(5). Our primary focus was to investigate the variations in gene expression patterns among Oligodendrocytes and Oligodendrocyte Progenitor Cells (OPCs) in between the different conditions.

After preprocessing with CellRanger, we performed quality control filtering, unsupervised graph-based clustering, and marker-gene cluster annotation with Seurat. We refined our clustering approach by comparing batch integration approaches. We applied fuzzy clustering strategies, differential gene expression, and marker gene analysis on individual cell type data subsets (e.g. oligodendrocytes). We performed both single-cell but also Pseudobulk DGE analysis and we complete our workflow with GSEA, active gene regulatory networks, and trajectory analysis.

Our results suggest that firstly the oligodendrocyte and OPC populations are affected in both C9orf72 and Sporadic ALS, compared to the controls. We identify clusters of cells, that express most of the DEG found to be disease-correlated, like NDUFA4, CCK, and others. In this cluster, the highest percentage of the cells are derived from the disease samples. Thus we assume that these clusters should be affected most by the disease. In the Motor Cortex, the differences in the transcription profiles were more obvious in the C9orf72 compared to sALS group. On the other side, in the Spinal Cord, the transcription profiles of both C9orf72 and Sporadic ALS were altered compared to the controls. The main pathways dysregulated in ALS patients include mitochondrial, ribosomal, and proteasome function, ubiquitination, and metal (mainly iron and copper) metabolism. In the end, many of the dysregulated mRNAs are known as TDP-43 targets. Our results suggest that there is a clear involvement of oligodendrocytes and OPCs in ALS and open new avenues for further research on their role in the pathogenesis of ALS.



14. Untangling the role of microRNAs in ALS pathogenesis via the iPSC-derived skeletal muscle in vitro model derived from C9ORF72-mutant patients.

Claudia Malacarne (1)*, Erika Salvi (2), Eleonora Giagnorio (1), Fulvia Saraceno (3), Patrizia Bossolasco (4), Chiara Lattuada (4), Antonia Ratti (5), Giuseppe Lauria (6,7), Mantegazza (1), Silvia Bonanno (1), Stefania Marcuzzo (1)

(1) Neurology IV –Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

(2) Neuroalgology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

(3) University of Parma, Department of Chemistry, Life Sciences and Environmental Sustainability, Parma, Italy.

(4) Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy.

(5) Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy; Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy.

(6) Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

(7) Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which the C9ORF72 repeat expansion is currently the leading genetic cause of both hereditary and sporadic cases.

The lack of a cure highlights the need to promptly dig deeper into the underpinning pathological mechanisms.

Growing evidence indicates that early alterations occur in ALS muscle cells, suggesting a crucial role of skeletal muscle in the disease. However, these events have not been fully investigated yet, especially in the C9ORF72-ALS pathology.

MicroRNAs (miRNAs) are key molecules involved in several pathways, including myogenesis, in both physiological and disease conditions such as ALS.

The aim of the study is to explore miRNA role in a human iPSC-derived skeletal muscle in vitro model to uncover novel therapeutic targets for C9ORF72-associated ALS.

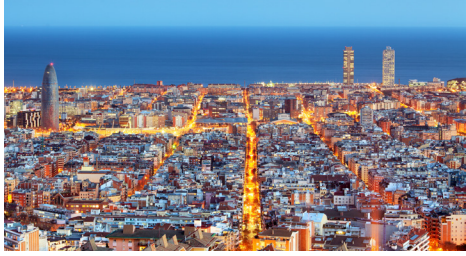
By a small molecule-based approach, we differentiated iPSCs derived from 3 C9ORF72 sporadic patients and 3 healthy controls into myotubes. The iPSC-derived myotubes were characterized by molecular analysis and immunofluorescence. Next, using microfluidic Taqman Array cards a miRNome profiling of 754 miRNAs was performed in C9ORF72 and control myotubes.

The obtained mature C9ORF72 iPSC-derived myotubes displayed an early contractile activity, in line with molecular data showing a differential expression of myogenic markers – i.e. MyHC, MYOG, and DESMIN – compared to controls. Interestingly, the GGGGCC expansion was retained in all lines after reprogramming and differentiation, and nuclear and cytoplasmic C9ORF72 RNA foci were detected by in situ RNA analysis.

Moreover, the miRNome profiling revealed four miRNAs exclusively expressed in control cells compared to the C9ORF72 lines. By miRTarBase and Tarbase in silico screening and enrichment analyses, we demonstrated that the dysregulated miRNAs could have a crucial involvement in muscle tissue development, RNA and protein metabolic process, and nuclear transport, suggesting a key function of these molecules in C9ORF72-associated muscle differentiation impairment.

Overall we demonstrated that our iPSC-derived muscle in vitro model is a reliable patient-specific tool to untangling miRNA role in the C9ORF72-related muscle pathology underlying ALS.

Further studies are needed to pave the way for novel and tailored therapeutic strategies targeting skeletal muscle, based on miRNA modulation.



15. Sex-stratified analysis of ~133k samples identifies novel associations with Amyotrophic Lateral Sclerosis

Ross P Byrne (1*), Wouter van Rheenen (2), Jan H Veldink (2), Russell L McLaughlin (1)

1. Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland.

2. Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht, The Netherlands.

Background:

ALS shows both higher incidence and prevalence in males, suggesting sex is a factor in disease risk. Sex hormones play a role in this, with evidence that male sex hormones increase risk and female sex hormones decrease risk. However, recently observed differences in ALS heritability between males and females suggests genetics may also play a role in the sex differences observed in ALS.

Objective:

Our study aims to identify sex-specific genetic variants acting as risk factors for ALS through sex-stratified analysis of the European samples from the latest ALS GWAS dataset (N~133k).

Methods:

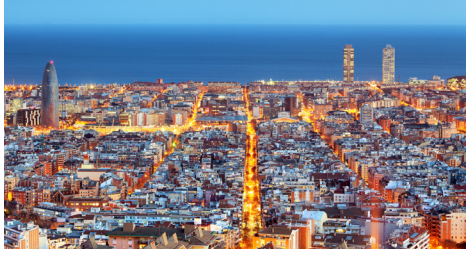
We ran SAIGE on male-only (N=65,692) and female-only (N=67,957) strata to generate sex-stratified GWAS summary statistics. To test for global differences in genetic architecture, genetic correlation between sexes was estimated with LD score regression. We performed genome-wide scans for sex-specific variants, defined as variants passing a 1% FDR threshold for association in one sex with no evidence of association in the other (p>0.05). Finally we integrated expression data for 13 brain tissues from GTEx with our sex-specific GWAS statistics in a transcription wide association study (TWAS). This tested for association between gene expression in brain tissues and ALS in males and females.

Results:

Despite near perfect genetic correlation between sexes (LD score regression: rg=0.99; se=0.16), sex-specific genome-wide scans identified significantly associated variants in several novel male-specific (WIPI2, LUZP2 and RESP18) and female-specific (FGFRL1 and MEF2C) loci. Additionally, while not sex-specific by our definition, both SCFD1 and the HLA locus reached genome-wide significance only in males. TWAS analysis revealed significant sex-specific associations between expression of two of these genes (MEF2C and RESP18) in brain tissues and ALS.

Discussion:

Among our novel sex-specific associations MEF2C shows a particularly promising link to ALS. MEF2C is a transcription factor that has been shown to be upregulated in sporadic and SOD1+ ALS patients. MEF2C notably regulates MECP2, a gene on the X-chromosome involved in nerve cell function and implicated in the neurodevelopmental disorder Rett syndrome. Regulation of MECP2 may explain MEF2C's female specific effect, motivating further study of the X chromosome in ALS. Thus, our analysis reveals several novel sex-specific associations with ALS supported by tissue specific expression and existing literature.



16. Genome-wide methylation array reveals epigenetic drift and epivariations in ALS

Alberto Brusati(1*), Antonia Ratti(2,3), Luciano Calzari(4), Silvia Peverelli(2), Cinzia Tiloca(2), Erika Carbone(4), Vincenzo Silani(2,5), Davide Gentilini(1,4), Nicola Ticozzi(2,5)

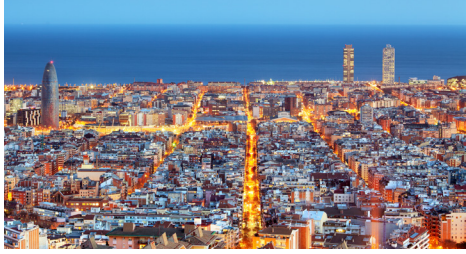
1 - Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy.
2 - Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy.
3 - Department Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.
4 - Bioinformatics and Statistical Genomics Unit,IRCCS Istituto Auxologico Italiano,Milan,Italy.
5 - Department of Pathophysiology and Transplantation, Dino Ferrari Center, University of Milan, Milan, Italy.

During the last decades, our knowledge about the genetic architecture of sporadic ALS has significantly increased. However, besides the recognized genetic risk factors, also the environment is supposed to have a role in disease pathogenesis. Epigenetics, reflecting the direct consequences of the interaction between genes and environmental risk factors, may play a role in the development and progression of ALS. A recent large epigenome-wide association study (EWAS) in blood identified differentially methylated positions mapping to 42 different genes, involved in cholesterol biosynthesis and immune-related pathways.

Here we performed a genome-wide DNA methylation analysis in whole peripheral blood on an Italian cohort of 61 sporadic ALS patients and 61 healthy controls, sex- and aged-matched. We found an increased epigenetic drift in ALS cases compared to controls. By a meta-analytical approach, we conducted a gene set enrichment analysis (GSEA) on significant concordant probes with those obtained in the previous EWAS on ALS. By using the resulting genes, we investigated their relationship with toxic compounds according to the Toxicogenomic Database. Moreover, for the first time, we calculated regions enriched in stochastic epigenetic mutations (SEMs), also named epivariations. Interestingly, we identified epivariations in 8 genes expressed in cerebral areas, uniquely enriched in ALS cases compared to controls and associated with neural impairments in literature.

Overall, our study reinforces the evidence that epigenetics may contribute to the pathogenesis of ALS and that epigenetic drift may be a useful diagnostic marker. Further research is needed to determine the role of epivariations in the identified candidate genes.

Session 5. Therapeutics



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

Loreto Martinez-Gonzalez* (1,2), Carmen P de la Lastra-Aranda (1), Marta Gomez-Almeria (3), Eva de Lago (2,3), Valle Palomo (2,4), Ana Martinez (1,2)

1) Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain.
2) Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain.
3) Facultad de medicina, Universidad Complutense de Madrid, Spain.
4) IMDEA Nanociencia, Madrid, Spain.

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

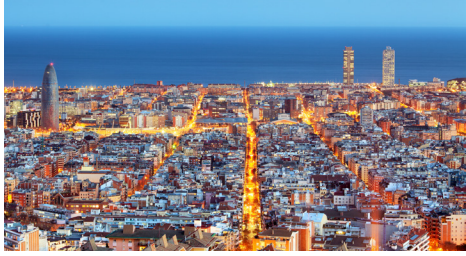
The small brain penetrant molecule known as IGS2.7 is a potent and selective protein kinase CK-1 inhibitor that exerts its neuroprotective effect by reducing TDP-43 hyperphosphorylation, reactive gliosis and consequently motor neuron death in a transgenic TDP-43 mouse model. In sporadic ALS immmortalized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

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

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

1) Martínez-González, L., Rodríguez-Cueto, C., Cabezudo, D. et al. Motor neuron preservation and decrease of in vivo TDP-43 phosphorylation by protein CK-1δ kinase inhibitor treatment. Sci Rep 10, 4449 (2020).

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



18. The MIROCALS Study: Efficacy of low dose IL2 in ALS and implications for ALS trial design.

P.Nigel Leigh (1)*, Gilbert Bensimon (2), Payan C (2), Ammar Al-Chalabi (3), Ulf Andreasson (4), Brian Dickie (5), Cecilia Garlanda (6), Janine Kirby (7), Pieter Klaassens ((8), Massimo Locati (6), Andrea Malaspina (9), Christophe Masseguin (10), Claudie Muller (2), Phuong Huang Pham (11), Safaa Saker-Delye, (12) Pamela Shaw (7), Timothy Tree (13), Henrik Zetterberg (4) and the MIROCALS Trial Consortium.

1.Department of Neuroscience, Brighton and Sussex Medical School, Falmer, UK.

2. Centre Hospitalier Universitaire de Nîmes and Clinical Pharmacology Department, Hôpital Pitié-Salpétrière, Paris, France.

3. Department of Clinical and Basic Neuroscience, Institute of Psychiatry Psychology and Neuroscience, King's College London, UK.

4. Department of Psychiatry and Neurochemistry, University of Gothenburg, Göteburg, Sweden.

5. Motor Neurone Disease Association of England Wales and Northern Ireland, Northampton, UK.

6. Humanitas Mirasole SPA, Rozzano, Italy.

7. Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK.

8. WGK Clinical Services Ltd, Luton, Bedfordshire, UK.

9.Department of Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery and Institute of Neurology, University College London, UK.

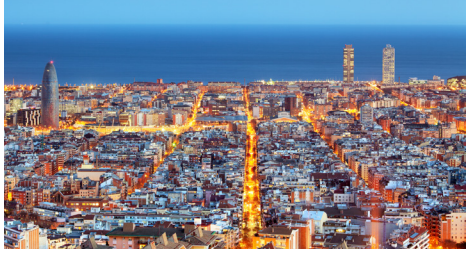
10. Centre Hospitalier Universitaire de Nîmes, Nimes, France.

11. Parean biotechnologies, Saint-Malo, France.

12. Généthon, Evry-Courcouronnes, France.

13. Department of Immunobiology, King's College London, London, UK.

Our phase IIA trial ('IMODALS') demonstrated that low dose (ld) IL2 treatment boosted regulatory T cells (Tregs) and decreased blood inflammatory markers indicating a possible role in ALS therapy. Objective: Modifying Immune Responses and Outcomes in ALS (MIROCALS:NCT 03039673) was a phase IIB trial of the efficacy of ld-IL2 in ALS. Volunteers with newly diagnosed ALS, naïve to riluzole, were randomised 1:1 to ld IL2 (2 million units) or placebo in 5-day cycles every 28 days over 18 months by subcutaneous injection after a 3 month run-in on riluzole prior to randomisation. Blood and CSF samples were collected at baseline, randomisation, and week 17 post-randomisation. Pre-specified core biomarkers were CSF phosphorylated neurofilament (pNFH), CSF CCL2, and blood Tregs. The primary efficacy outcome was time to death at 21 months (640 days) post-randomisation in the Intent-To-Treat (ITT) population. Treatment effect on survival was analysed by unadjusted (log-rank) analysis and (pre-specified) adjustment for major prognostic covariates (Cox model). Main secondary efficacy outcomes were safety and ALSFRS-R slope of change. Results. 220 people with ALS were recruited in 17 ALS Centres in UK (7 centres) and France (10 Centres). Demographic variables were well-balanced across treatments. There was no loss to follow-up at 21 months.Treg responses showed effective target engagement. Unadjusted log-rank analysis of survival showed a non-significant 19% reduction in risk of death with IL2 compared to placebo at 21 months but pre-specified analysis adjusted on CSF pNFH in the ITT population showed a statistically significant (73%) reduction in risk of death (hazard ratio 0.27; p=0.0017). Joint rank analysis of ALSFRS-R slope of change adjusted on CSF pNFH showed a significant decrease in rate of change in ALSFRS-R slope with IL2 compared to placebo. No treatment-related change was detected in plasma or CSF NF levels. Conclusion: Using an 'experimental medicine' design with biomarkers in blood and CSF we show that low-dose IL2 as add-on to riluzole reduced the risk of death at 21 months after 18 months treatment. MIROCALS was funded by the European Union H2020 programme, The PHRC, The MND Association, Association Francaise Contre les Myopathies (AFM), MND Scotland, MyName'5Doddie Foundation, Association pour la Recherche sur La SLA, and the Alan Davidson Foundation. ** https://symposium.mndassociation.org/wp-content/uploads/2022/10/The-MIROCALS-Study-Group.pdf



19. Lys-acetylated PPIA as a candidate therapeutic target for TDP-43 proteinopathies

Laura Pasetto* (1), Serena Scozzari (1), Stefano Fabrizio Columbro (1), Serafina Spagnolo (1), Giovanni De Marco (2), Martina Greco (3), Andrea Calvo (2), Manuela Basso (3), Valentina Bonetto (1).

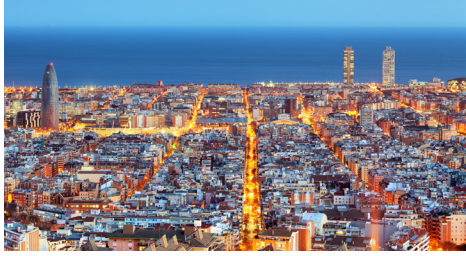
(1) Research Center for ALS, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano, Italy.
(2) "Rita Levi Montalcini" Department of Neuroscience, University of Torino, Torino, Italy.
(3) Dipartimento di Biologia Cellulare, Computazionale e Integrata, CIBIO, Trento, Italy.

Up to 97% of amyotrophic lateral sclerosis (ALS) patients and about 45% of patients with frontotemporal lobar degeneration present TDP-43 inclusions, suggesting an essential role for this protein in disease pathogenesis. Gain of cytoplasmic functions and loss of nuclear functions of TDP-43 seem to contribute to pathogenesis and impairment in its trafficking be a key event. We demonstrated that peptidyl-prolyl cis-trans isomerase A (PPIA), also known as cyclophilin A, interacts with TDP-43 and regulates its trafficking and function. PPIA knockout (PPIA-/-) mice develop a neurodegenerative disease which resembles ALS-frontotemporal dementia (FTD) with marked TDP-43 pathology. We found that the interaction between TDP-43 and PPIA depends on PPIA acetylation at K125 (acetyl-PPIA), which is impaired in experimental models of ALS and in patients, where low level of acetyl-PPIA is associated with TDP-43 mislocalization. We are now evaluating if increasing acetyl-PPIA is a promising therapeutic strategy by two different approaches: (a) pharmacologically with HDAC inhibitors (HDACi) and (b) delivering an acetyl-PPIA mimetic by an AAV9-based gene therapy. We are testing the effects of these treatments in clearing TDP-43 proteinopathy in vitro and in vivo models of ALS and ALS/FTD.

We found that PPIA is mainly de-acetylated by HDAC1 by silencing different HDACs in HEK293 cells and evaluating the effect on PPIA acetylation. Treatment with inhibitors of class I HDACs increased acetyl-PPIA levels and reduced TDP-43 mislocalization in a cellular model of TDP-43 pathology and in PBMCs of ALS patients. HDACi treatment in the TAR4/4 TDP-43 mouse model of ALS at an early stage of the disease attenuated TDP-43 pathology, exerted a neuroprotective effect and induced a delay in symptom onset.

K to Q substitution mimics acetyl-K for similarity in chemical properties. In a cellular model of TDP-43 pathology, transfection with the acetyl-PPIA mimetic, PPIAK125Q, substantially reduced insoluble phosphorylated TDP-43 compared to wild-type PPIA or the acetylated-defective mutant PPIAK125R. We are now investigating the AAV9-mediated PPIAK125Q gene delivery to neurons and motor neurons in the TAR4/4 TDP-43 mouse model of ALS.

Our findings suggest that increasing acetyl-PPIA is possibly an effective therapeutic strategy for TDP-43 proteinopathies.



20. Neurofilament light chain response during therapy with Tofersen in SOD1-related ALS – treatment experience in clinical practice.

Thomas Meyer (1,2), Peggy Schumann (2), Patrick Weydt (3,4), Susanne Petri (5), Yasemin Koc (1), Susanne Spittel (2), Sarah Bernsen (1,3), René Günther (6, 7), Jochen H. Weishaupt (8), Marie Dreger (1), Felix Kolzarek (2), Dagmar Kettemann (1), Jenny Norden (1), Matthias Boentert (9), Maximilian Vidovic (6), Christian Meisel (10,11), Christoph Münch (1,2), André Maier* (1), Péter Körtvélyessy* (1,12)

1) Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Neurology, Center for ALS and other Motor Neuron Disorders, Berlin, Germany

2) Ambulanzpartner Soziotechnologie APST GmbH, Berlin, Germany

3) Bonn University, Department for Neurodegenerative Disorders and Gerontopsychiatry, Bonn, Germany

4) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Bonn, Germany

5) Hannover Medical School, Department of Neurology, Hannover, Germany

6) Technische Universität Dresden, University Hospital Carl Gustav Carus, Department of Neurology, Dresden, Germany

7) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Dresden, Germany

8) University Medicine Mannheim, Heidelberg University, Mannheim Center for Translational Medicine, Neurology Department, Division for Neurodegenerative Diseases, Mannheim, Germany

9) Münster University Hospital, Department of Neurology, Münster, Germany

10) Labor Berlin - Charité Vivantes GmbH, Department of Immunology, Berlin, Germany

11) Charité – Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Berlin, Germany

12) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Magdeburg, Germany

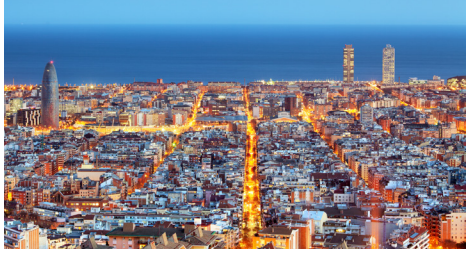
**Contributed equally*

Introduction/Aims: In amyotrophic lateral sclerosis (ALS) caused by superoxide dismutase 1 (SOD1) gene mutations (SOD1-ALS), the antisense oligonucleotide tofersen had been investigated in a phase 3 study (VALOR) and subsequently introduced in an expanded access program. This study assesses neurofilament light chain (NfL) before and during tofersen treatment.

Methods: In six SOD1-ALS patients treated with tofersen at three specialized ALS centers in Germany, NfL in cerebrospinal fluid (CSF-NfL) and/or serum (sNfL), the ALS Functional Rating Scale–Revised (ALSFRS-R), and ALS progression rate (ALS-PR), defined by monthly decline of ALSFRS-R, were investigated.

Results: Three of six SOD1-ALS patients reported a negative family history. Three patients harbored a homozygous c.272A>C, p.(Asp91Ala) mutation. These and two other patients showed slower progressing ALS (defined by ALS-PR <0.9) whereas one patient demonstrated rapidly progressing ALS (ALS-PR=2.66). Mean treatment duration was 6.5 months (range 5-8). In all patients, NfL decreased (mean CSF-NfL -66%, range -52 to -86%, mean sNfL -62%, range -36 to -84%). sNfL at 5 months of tofersen was significantly reduced compared to the measurement closest before treatment (p=0.017). ALS-PR decreased in two patients whereas no changes in ALSFRS-R were observed in four participants who had very low ALS-PR or ALSFRS-R values before treatment.

Discussion: In this case series, the significant NfL decline following tofersen treatment confirmed its value as a response biomarker in an expanded clinical spectrum of SOD1-ALS. Given the previously reported strong correlation between sNfL and ALS progression, the NfL treatment response contributes to the notion of disease-modifying activity of tofersen.



21. Evaluating single and multiple ascending doses of WVE-004 in C9orf72-associated ALS and FTD: results from the FOCUS-C9 Trial

Michael Tillinger1, Merit Cudkowicz2, Jonathan D. Rohrer3, Leonard H. van den Berg4, Johnathan Cooper-Knock5, Simon Ducharme6, Kenechi Ejebe7, Andrew Hart1, Yili Pritchett1, Xiao Shelley Hu1, Padma Narayanan1, Maneet Singh1, Stephen L. Lake8, Michael Panzara9, Anne-Marie Li-Kwai-Cheung1

(1) Wave Life Sciences, Cambridge, MA, USA

(2) Sean M Healey & AMG Center for ALS at Massachusetts General Hospital, Boston, MA, USA

(3) Dementia Research Centre, University College London Queen Square Institute of Neurology, London, UK

(4) Department of Neurology, University Medical Centre Utrecht Brain Centre, Utrecht University, Utrecht, The Netherlands

(5) Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield S10 2HQ, UK

(6) Montreal Neurological Institute and Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada

(7) Kenechi Ejebe was an employee of Wave Life Sciences during the design and conduct of the study.

(8) Alexion Pharmaceuticals, Boston, MA, USA. Stephen L. Lake was an employee of Wave Life Sciences during the design and conduct of the study.

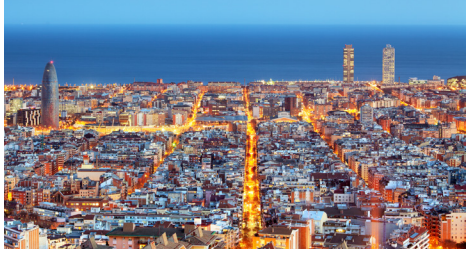
(9) Neurvati Neurosciences, New York, NY, USA. Michael Panzara was an employee of Wave Life Sciences during the design and conduct of the study.

WVE-004 is an investigational stereopure antisense oligonucleotide designed to selectively target pathological C9orf72 transcripts while sparing C9orf72 protein. FOCUS-C9 (NCT04931862) was a global Phase 1b/2a trial designed to assess the safety and tolerability, as well as the pharmacodynamic, pharmacokinetic, and clinical effects of single- and multiple-ascending intrathecal doses of WVE-004 in people with C9orf72-ALS and -FTD.

In the single-ascending dose portion of this study, patients were randomized to 4 dose cohorts (10 mg, n=2; 20 mg, n=8; 30 mg, n=5; 60 mg, n=3) and placebo (n=5). In the multidose portion of the study, 10 patients were continued from the single-dose period while 12 new patients were enrolled (n=22; 10 mg monthly, n=6; 10 mg quarterly, n=9; placebo, n=7). Three patients treated with placebo in the single-dose portion of the study continued with placebo in the multidose portion. WVE-004 was generally safe and well-tolerated across doses. Most adverse events (AEs) presented as mild in intensity and were related to disease progression or intrathecal administration.

In this planned analysis, we confirmed that WVE-004 leads to robust and sustained reductions in poly(GP) in CSF from baseline, with a maximal mean reduction of 51% in the 20 mg single dose cohort at Day 169 (p=0.0006), of 48% in the quarterly 10 mg dose (p<0.0001) at Day 113, and 50% (p=0.0001) in the monthly 10 mg dose at Day 169. In the 10 mg monthly cohort, mean decline in ALSFRS-R was statistically significantly greater than the placebo group at week 24 (p=0.0009), but these changes were not statistically different from a propensity score-matched natural history cohort from the PRO-ACT database. In the quarterly cohort, there was no difference in ALSFRS-R mean change between WVE-004 and placebo or the propensity score-matched natural history cohort at any timepoint through 24

Session 6. Neuropathology and applied clinical neuroscience



22. Metabolic Alterations precede Neurofilament Changes in Presymptomatic ALS Gene Carriers

Johannes Dorst(1,2)*, Patrick Weydt(3)*, David Brenner(1,2), Simon Witzel(1), Katharina Kandler(1), André Huss(1,2), Christine Herrmann(1), Maximilian Wiesenfarth(1), Antje Knehr(1), Kornelia Günther(1), Kathrin Müller(4), Jochen H Weishaupt(5), Johannes Prudlo(6), Karin Forsberg(7), Peter M Andersen(7), Angela Rosenbohm(1), Joachim Schuster(1,2), Francesco Roselli(1,2), Luc Dupuis(8), Benjamin Mayer(9), Hayrettin Tumani(1,2), Jan Kassubek(1,2), Albert C Ludolph(1,2)

(1) Department of Neurology, University of Ulm, Ulm, Germany.

(2) German Center for Neurodegenerative Diseases (DZNE), Ulm, Germany.

(3) Department of Neurodegenerative Disease and Gerontopsychiatry/Neurology, University of Bonn Medical Center, Bonn, Germany.

(4) Institute for Human Genetics, University of Ulm, Ulm, Germany.

(5) Division of Neurodegenerative Disorders, Department of Neurology, Medical Faculty Mannheim, Mannheim Center for Translational Neurosciences, Heidelberg University, Mannheim, Germany.

(6) Department of Neurology, Rostock University Medical Center, and German Center for Neurodegenerative Diseases (DZNE), Rostock, Germany.

(7) Department of Clinical Science, Neurosciences, Umeå University, Umeå, Sweden.

(8) Inserm, Université de Strasbourg, Strasbourg, France.

(9) Institute for Epidemiology and Medical Biometry, University of Ulm, Ulm , Germany.

Background

The emergence of potentially effective new therapies for genetic forms of amyotrophic lateral sclerosis (ALS) necessitates the identification of biomarkers to facilitate early treatment, prior to the onset of motor symptoms. Here, we sought to investigate whether metabolic alterations are detectable in presymptomatic ALS gene mutation carriers, and whether such alterations precede neurofilament light chain (NfL) changes in serum.

Methods

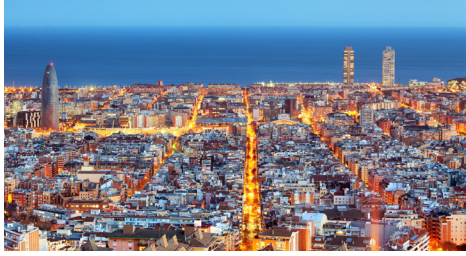
Between 02/2014 and 11/2021, we prospectively studied 60 presymptomatic ALS gene mutation carriers (40% male, age 48.7 ± 14.9; 28 C9orf72, 22 SOD1, 10 other) compared to 73 individuals from the same families (47% male, age 47.4 ± 12.9) without pathogenic mutations as controls. Bioimpedance analysis (BIA) and indirect calorimetry were performed, and Body Mass Index (BMI), Fat Mass (FM), Body Fat Percentage, Body Water (BW), Lean Body Mass (LBM), Extracellular Mass (ECM), Body Cell Mass (BCM), ECM/BCM ratio, Cells Percentage, Phase Angle, Resting Metabolic Rate (RMR), Metabolic Ratio (MR), and NfL were measured. Participants and evaluators were blinded regarding gene carrier status.

Findings

Presymptomatic ALS gene carriers showed reduced LBM (p=0.02), BCM (p=0.004), Cells Percentage (p=0.04), BW (p=0.02), Phase Angle (p=0.04), and increased ECM/BCM ratio (p=0.04), consistently indicating a loss of metabolically active body cells. While in C9orf72 mutation carriers all tissue masses were reduced, only metabolically active tissue was affected in SOD1 mutation carriers. Unexpectedly, RMR (p=0.009) and MR (p=0.01) were lower in presymptomatic ALS gene carriers compared to non-carriers. NfL serum levels were similar in mutation carriers and non-carriers (p=0.60).

Interpretation

The observed metabolic phenomena might reflect reduced physical activity and/or preemptive, insufficient compensatory mechanisms to prepare for the later hypermetabolic state. As pre-symptomatic biomarkers we propose ECM/BCM ratio and Phase Angle for SOD1, and a 4-compartment affection in BIA for C9orf72 mutation carriers.



23. Disruption of the Angiopoietin-like protein system correlates with lipid homeostasis in ALS

Sruthi Sankari Krishnamurthy*(1,2), Diana Wiesner(3), Veronika Klose(3), Stefano Antonucci(1), Natalie Yashoda Dikwella(1), Gizem Yartas(1,3), Dagmar Schattauer(1), Max Wiesenfarth(1), Ulrike Weiland(1),Tobias Boeckers(2,4), Luc Dupuis(5), Albert Ludolph(1,3), Hans-Peter Müller(1), Jan Kassubek(1), Johannes Dorst(1,3), Francesco Roselli(1,3)

1) Department of Neurology, Ulm University, Ulm (DE).

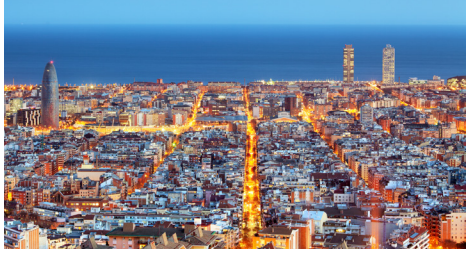
2) CEMMA graduate school, Ulm University, Ulm (DE).

3) German Center for Neurodegenerative Diseases (DZNE)-Ulm (DE).

4) Institute of Anatomy and Cell Biology, Ulm University, Ulm (DE).

5) Mécanismes Centraux et Périphériques de la Neurodégénérescence, Inserm, CRBS, Université de Strasbourg, Strasbourg (FR).

Dysregulated lipid metabolism is increasingly reported in ALS patients. Blood levels of triglycerides, cholesterol, LDL/HDL ratio are altered in ALS patients and presymptomatic gene carriers, but mechanistic insights into these changes are lacking. Angiopoietin like proteins (ANGPTLs) 3, 4 & 8 inhibit Lipoprotein lipase (LPL), accumulating triglyceride rich lipids in circulation. Measuring ANGPTLs in serum samples of healthy controls and ALS patients (cohorts of non-genetic, SOD1, C9orf72, FUS/TARDBP), revealed a mutation specific profile – ANGPTL3 & 4 were decreased in SOD1 and C9 patients compared to controls, with no change in FUS. Total serum cholesterol was reduced in the SOD1 group and increased in C9 patients. Measuring the activity of LPL in these samples, reaffirmed the inverse correlation between LPL and ANGPTLs. Interestingly, female non-genetic ALS patients (n=60) are characterized by higher ANGPTL3 levels than males, independently of their age or BMI status. Strikingly, a significant positive correlation was observed between systemic ANGPTL3 levels and hypothalamic volume measured via MRI in 66 ALS patients, establishing a novel connection between the central and peripheral systems in ALS, with the hypothalamus playing a key role in regulating metabolism. We then took advantage of the SOD1G93A murine model to understand the ANGPTL axis further. Systemic ANGPTL3,4 levels were lower than WT mice at the late symptomatic stage while, surprisingly, total cholesterol was already reduced at presymptomatic stage. To understand the source of the disrupted Angptls, transcription in peripheral tissues (liver, kidney, white and brown (BAT) adipose tissue, gastrocnemius) was studied by qPCRs. Angptl3 and Angptl4 were significantly reduced in BAT at the presymptomatic and late symptomatic stages respectively, at both gene and protein levels. Exploration of additional modulators of BAT function using an antibody array, unveiled alterations in 10 out of 38 adipokines, even in presymptomatic ALS mice. Additionally, abundant transcription of beta-adrenergic receptors 2 and 3 in BAT correlated to Angptls. This prompted a systemic beta-adrenergic stimulation study in presymptomatic SOD1 mice which normalized the levels of Angptl3 & 4 in BAT. We present converging evidence from human patients and murine models of an altered ANGPTL system in ALS; this finding constitutes one of the first molecularly defined entry points to manipulate lipid metabolism in ALS.



24. High-resolution assessment of ALS neuropathology and its association with clinical presentation

Anna Sanchez Avila (*)(1,2), Tara Spires-Jones (2,3), Thomas Gillingwater (2,3), Christopher Henstridge (1,2)

1 University of Dundee, Dundee, UK.
2 Euan MacDonald Centre for MND Research, Edinburgh, UK.
3 University of Edinburgh, Edinburgh, UK.

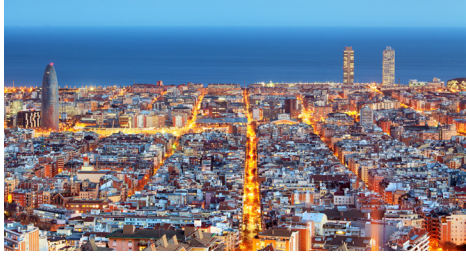
Half of ALS patients display cognitive impairment and have a worse prognosis and faster disease progression. Previously, synapse loss in the dorsolateral prefrontal cortex (BA9) has been associated with cognitive decline, but the regional specificity of synaptic degeneration and its correlation with the presence of symptoms remains to be assessed. Here, we build on our previous work to generate a database of comprehensive patient information including detailed clinical cognitive profiling, post-mortem high-resolution synapse density measurements as well as pathology presence and astrocytic and microglial burden.

We have studied Brodmann area BA44/45 (Broca's area), associated with language function, and Brodmann area BA17/19 (visual cortex), thought to be spared in ALS. This analysis has been performed in the same donors as our previous work, which means we have synapse density data from BA9, BA4 (motor cortex), BA44/45 and BA17/19 from each donor, collected using both electron microscopy and array tomography.

ALS patients were stratified based on their cognitive profile derived from their Edinburgh Cognitive and Behavioural ALS Screen (ECAS) scores, as well as the presence of a hexanucleotide repeat expansion in C9ORF72. Lastly, we have combined our synaptic-level analyses with cortical thickness measures as well as regional neuropathology analysis (presence of TDP-43, Tau or Amyloid), astrocytic and microglial burden as well as their correlation with demographic information on each individual donor.

Surprisingly, we observed no difference in synaptic density in either of the two areas studied, regardless of cognitive status, contrary to what was found in BA9. However, we found a decrease in cortical thickness in BA44/45 and BA17/19, suggesting a more advanced pathology and neuron loss. The cortical thinning in Broca's area seems to be exclusive to patients with cognitive decline, whereas cortical thinning in the visual cortex was independent of cognitive impairment or C9 status. No outright differences were seen in microglia or astrocytic burden between groups. We are in the process of performing correlation matrixes with the demographic information.

In summary, we have unique human dataset combining detailed cognitive assessment, regional neuropathology, and single synapse analysis to try and uncover the underlying pathology associated with cognitive decline in ALS.



25. Investigating the role of autoantibodies against neurofilaments in amyotrophic lateral sclerosis (ALS)

Ellie Sturmey* (1), Fabiola Puentes (1), Agnes Nishimura (1), Angray Kang (1), Andrea Malaspina (1,2)

1. Department of Neuroscience, surgery and trauma, Blizard Institute, Queen Mary University of London, London, United Kingdom.

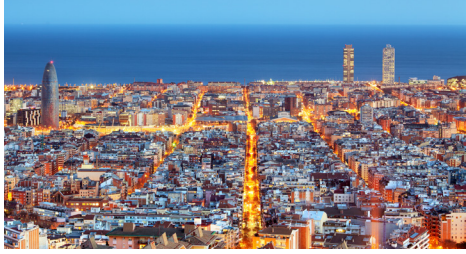
2. Queen Square Institute of Neurology, University College London, London, United Kingdom.

Background and objectives: Our lab previously reported that levels of circulating autoantibodies against neurofilaments (Nf-Abs) are elevated in ALS patients, with the greatest increase in advanced and fast-progressing disease (ALS-F) (Puentes et al., 2014, 2021). Unlike Nf levels, Nf-Ab levels continue to increase with disease progression. Considering this, and the well-established dysregulation of T-cells and subsequent loss of immune tolerance in ALS, we hypothesize that there may be a pathological burden associated to Nf-Abs in ALS, particularly in late-stage disease. In early disease, Nf-Abs may be protective (Couratier et al., 1998). In this study, we investigate the Nf-Ab response in ALS and their possible role in disease pathology. Given their greater stability in blood to Nfs, analysis of an array of Nf-Abs may also lead to establishing new clinically useful ALS biomarkers.

Methods: IgG from healthy control (HC), slow-progressing ALS (ALS-S) and ALS-F plasma have been affinity-purified using Protein G agarose. From this, we have purified IgG with Nf affinity using immobilized antigens. A naïve single-chain variable fragment (scFvs) DNA library has also been produced from ALS-F patient RNA. Via ribosome display, scFvs against proteins of interest can be selected (Tang et al., 2012). Functional testing of physiological IgG and of library-derived scFvs is currently being carried out in neuronally differentiated PC12 cells and isogenic iPSC cells (with ALS-linked mutations in TDP-43 and FUS). Initial tests involve measuring altered mitochondrial activity via MTT assay.

Results: Anti-rubella (control), anti-NfH and anti-NfL scFv DNA has been selected from the ALS-F naïve library, using a single round of ribosome display. Bacterial cloning was performed and positive colonies - identified by luciferase assay - had their plasmid DNA extracted and sent for Sanger sequencing. Following analysis of these sequences, clones were selected for protein expression in BL21 (DE3) E. coli. Selection of these antibodies has validated the PCR-generated library, which can be used now to generate scFvs to any protein of interest or to create higher affinity Nf-Abs via increased rounds of ribosome display.

Future steps: In the next couple of months, we plan to have functionally tested both the physiological IgG and library-derived scFvs. In doing so, we will ascertain whether there are functional differences between HC, ALS-S and ALS-F Nf-Abs, and/or between individuals.



26. Lipid-mediated resolution of inflammation in amyotrophic lateral sclerosis informs on novel biomarkers and therapeutic targets.

Ozlem Yildiz (1,2), Guy Hunt (3-6), Johannes Schroth (7), Tom Spargo (4), Ammar Al-Chalabi (4, 8), Michael R Barnes (9, 10), Martin R. Turner (11), Pamela J. Shaw (12),

Jesmond Dalli (13, 14), Sian Henson (7), Alfredo Iacoangeli (3, 4, 15), Andrea Malaspina (1,2)

1 Neuromuscular department, Motor Neuron Disease Centre, Queen Square Institute of Neurology, University College London, UK.

2 Neuroscience and Trauma, The Blizard Institute, Barts and The London School of Medicine & Dentistry, Queen Mary University of London, UK.

3 Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK.

4 Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK.

5 Perron Institute for Neurological and Translational Science, Nedlands, Australia.

6 Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Australia.

7 Translational Medicine and Therapeutics, William Harvey Research Institute, Barts and the London, Queen Mary University of London, UK.

8 Kingδ s College Hospital, London, UK.

9 Centre for Translational Bioinformatics, William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, London, UK.

10 The Alan Turing Institute, London, UK.

11 Nuffield Department of Clinical Neurosciences, University of Oxford.

12 Sheffield Institute for Translational Neuroscience, University of Sheffield.

13 William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK.

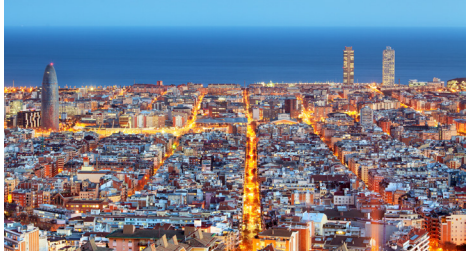
14 Centre for Inflammation and Therapeutic Innovation, Queen Mary University of London, UK.

15 National Institute for Health Research Biomedical Research Centre and Dementia Unit at South London and Maudsley NHS Foundation Trust and King's College London, UK.

Background: Circulating macrophage precursors and senescent lymphocytes are implicated in the altered systemic immune response unravelling in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder. Specialized pro-resolving mediators (SPMs) trigger the resolution of inflammation. We investigated SPMs blood profile and their receptors' expression in peripheral blood mononuclear cells (PBMCs) in relation to survival in ALS.

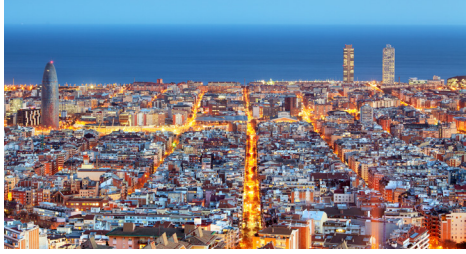
Methods: Patients living with ALS (pwALS) were stratified based on bulbar vs limb onset and on key progression metrics using a latent class model. SPMs plasma concentrations and their biosynthetic pathways were investigated by mass spectrometry at baseline and in one additional visit in 20 pwALS and 10 non-neurological controls (NNC; cohort 1). Flow cytometry was used to study resolvin receptors GPR32 and GPR18 expression in PBMCs from 40 pwALS and 20 NNC (cohort 2) at baseline and in two additional visits in 17 pwALS. Cox proportional–hazards models were applied to evaluate the effect of known clinical predictors and the mononuclear cells' expression of GPR32 and GPR18 on survival.

Results: Fast progressing (A-F) and bulbar onset (A-B) pwALS had increased concentrations of Resolvins (D1 & D6) in blood ($p<0.05$) and activated SPMs biosynthetic pathways compared to NCC. Resolvin receptors analysis in pwALS in AF, AB and fast progressing pwALS with bulbar onset A-FB) showed a GPR32 upregulation in monocytes expressing the active inflammation suppressing CD11b+ integrin. GPR32 and GPR18 were downregulated on several B and T cell subtypes except for upregulation of GPR18 in senescent late memory B cells, naive-Tregs and memory-Tregs ($p<0.01$). Additionally, GPR18 was upregulated in late senescent CD8+ T cells from most ALS phenotype variants compared to NCC ($p<0.01$). In survival models, expression of GPR32 and GPR18 on follicular B cells (HR:2.77 × 10^5, p= 0.000306) and



expression of GPR32 on intermediate monocytes (HR:2.59 × 10^40, p= 0.000146) impacted negatively on survival along with the known clinical predictors.

Conclusions: pwALS with the most severe disease phenotype have an increased blood expression of anti-inflammatory resolvins and differential regulation of resolvin receptors across all blood mononuclear cell subtypes, including senescent lymphocytes. These findings point to novel immunoregulatory strategies and biomarkers for ALS.



27. Unsupervised machine learning identifies distinct molecular and phenotypic ALS subtypes in post-mortem motor cortex and blood expression data

Heather Marriott* (1,2), Renata Kabiljo (2), Guy P Hunt (1,2,4,5), Ashley R Jones (1), Ahmad Al Khleifat (1), Claire Troakes (1,3), Project MinE ALS Sequencing Consortium, Abigail L Pfaff (4,5), John P Quinn (6), Sulev Koks (4,5), Richard JB Dobson (2,7,8,9), Patrick Schwab (10), Ammar Al-Chalabi (1,11) and Alfredo Iacoangeli (1,2,7)

1. Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

2. Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

3. MRC London Neurodegenerative Diseases Brain Bank, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

4. Perron Institute for Neurological and Translational Science, Australia.

5. Centre for Molecular Medicine and Innovative Therapeutics, Murdoch University, Murdoch, Australia.

6. Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, UK.

7. NIHR Maudsley Biomedical Research Centre (BRC) at South London and Maudsley NHS Foundation Trust and King's College London, London, UK.

8. Institute of Health Informatics, University College London, London, UK.

9. NIHR Biomedical Research Centre (BRC) at University College London Hospitals NHS Foundation Trust, London, UK.

10. GlaxoSmithKline, Artificial Intelligence and Machine Learning.

11. King's College Hospital, London, UK.

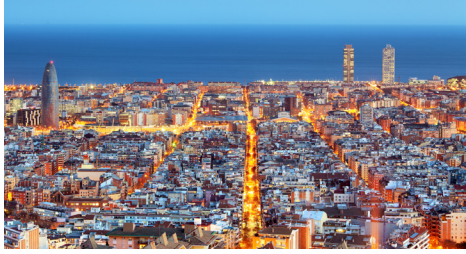
Background: Amyotrophic lateral sclerosis (ALS) displays considerable clinical, genetic and molecular heterogeneity. Machine learning approaches have already successfully been utilised for patient stratification in ALS, however, their lack of independent validation in different populations and ante-mortem tissue samples have greatly limited their use in clinical and research settings. We overcame such issues by performing a large-scale study of over 600 post-mortem brain and blood samples of people with ALS.

Methods: Hierarchical clustering was performed on the top 5000 most variably expressed autosomal genes identified from post-mortem motor cortex expression data of people with sporadic ALS from the KCL BrainBank (N=112). The molecular architectures of each cluster was established from gene enrichment, network and cell composition analysis. Methylation and genetic data was also used to assess if other omics measures differed between individuals. Validation of these clusters was achieved by applying linear discriminant analysis models to the TargetALS US motor cortex (N=93), and Italian (N=15) and Dutch (N=397) blood expression datasets. Sub-cluster phenotype analysis was also performed to assess cluster-specific differences in clinical outcomes.

Results: We identified three molecular phenotypes, which reflect the proposed major mechanisms of ALS pathogenesis; synaptic and neuropeptide signalling, excitotoxicity and oxidative stress, and neuroinflammation. Known ALS risk genes were identified among the informative genes of each cluster, suggesting potential for genetic profiling of the molecular phenotypes. Cell types which are known to be associated with specific molecular phenotypes were found in higher proportions in those clusters. These molecular phenotypes were validated in independent motor cortex and blood datasets with high accuracy (> 90%), with clinical phenotype analysis identifying distinct cluster-related outcomes associated with progression, survival and age of death, and were also found to be ALS-specific.

Conclusions: Our results support the hypothesis that each mechanism underlying ALS pathogenesis can present in subgroups of patients with a specific expression signature. These molecular phenotypes show potential to be utilised for the stratification of clinical trials, the development of biomarkers and for personalised treatment approaches.

Basic research Rapid Fire (1/2)



I) Deletion of endothelial TDP-43 disrupts the vascular barrier triggering inflammation and hemorrhages in the central nervous system

Victor Arribas* (1), Yara Onetti (1), Marina Ramiro (2,3), Pilar Villacampa (1), Ofelia Martinez (2,3), Heike Beck (4), Markus Sperandio (4), Bettina Schmid (5), Eloi Montanez (1)

1 Department of Physiological Sciences, Faculty of Medicine and Health Sciences, University of Barcelona and Bellvitge Biomedical Research Institute, 08907 L'Hospitalet del Llobregat, Barcelona, Spain

2 Celltec-UB, Department of Cell Biology, Physiology, and Immunology, Faculty of Biology, University of Barcelona, Barcelona, Spain

3 Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Spain

4 Walter Brendel Centre of Experimental Medicine, Biomedical Center, Institute of Cardiovascular Physiology and Pathophysiology, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany

5 German Center for Neurodegenerative Diseases (DZNE), Munich, Germany

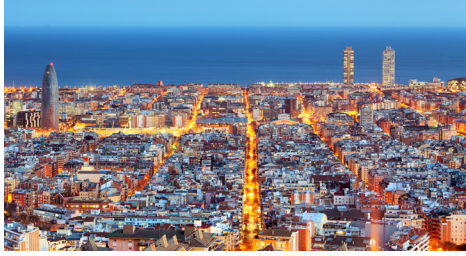
6 Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

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

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

In maturing vessels of the central nervous system, loss of TDP-43 results in altered actin cytoskeleton organization, disorganized distribution of cell-cell junction proteins and impaired vascular barrier integrity. and, consequently, hemorrhages and inflammation in the retina, brain and spinal cord. Cultured TDP-43-depleted ECs show reduced stable adherens junctions and altered cell-matrix adhesion sites. Mechanistically, loss of TDP-43 leads to increased actomyosin contraction, preventing proper formation of cell-cell and cell-matrix adhesions.

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



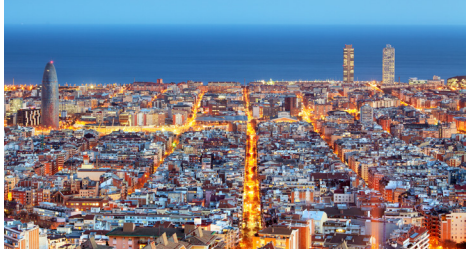
II) Haploinsufficiency of C9ORF72 selectively impairs autophagy in C9ORF72-linked ALS.

Rim Diab1,2,3, Federica Pilotto1,2,3, Niran Maharjan1,2, Shenyi Jiang4,5, Sabine Liebscher4,5,6, Andrea Salzinger4,5,6, Bhuvaneish T. Selvaraj7,8, Siddharthan Chandran7,8, Smita Saxena1,2*

1Department of Neurology, Inselspital University Hospital, Bern, Switzerland. 2Department for Bi-oMedical Research, University of Bern, Bern, Switzerland. 3Graduate School for Cellular and Bio-medical Sciences, University of Bern, Switzerland. 4Munich Cluster for Systems Neurology (SyN-ergy), Munich, Germany.5Institute of Clinical Neuroimmunology, Klinikum der Universität Mün-chen, Ludwig-Maximilians University Munich, Martinsried, Germany. 6Biomedical Center, Medi-cal Faculty, Ludwig-Maximilians University Munich, Martinsried, Germany.7UK Dementia Re-search Institute at University of Edinburgh, University of Edinburgh, Edinburgh EH16 4SB, UK. 8Euan MacDonald Centre for MND Research, University of Edinburgh, Edinburgh, EH16 4SB, UK.

*Corresponding author: Smita Saxena, Dept. of Neurology, Inselspital, Freiburgstrasse 16, CH-3010 Bern, Switzerland. Tel: +4131 632 13 47, email: smita.saxena@dbmr.unibe.ch

One of the most common genetic mutations associated with familial Amyotrophic lateral sclerosis (ALS) and Fronto-temporal dementia (FTD) has been identified as the hexanucleotide repeat expansion of GGGGCC (G4C2) in the intron of the gene chromosome 9 open reading frame 72 (C9ORF72). Although the underlying mechanisms of ALS onset are unknown, new insights implicate altered proteome homeostasis as a fundamental process underlying ALS pathogenesis. Motor neurons are intrinsically vulnerable to proteome stress. Unfolded or misfolded proteins are normally cleared by the various cell's clearance machinery such as the Ubiquitin proteasome system (UPS), ER-associated degradation and autophagy. C9ORF72 protein interacts with SMCR8 (Smith–Magenis syndrome chromosomal region candidate gene 8) and WDR41 (WD40 repeat-containing protein 41) to form stable CSW complex, which acts as a GDP/GTP exchange factor for Rab proteins, resulting in autophagy regulation. TANK Binding Kinase, TBK1, a serine-threonine kinase, phosphorylates SMCR8. Loss of function mutations in TBK1 were found to be associated with familial and sporadic ALS. Similarly, haploinsufficiency of C9ORF72 has been well established as one of the pathogenic mechanisms in C9ORF72-linked ALS. These findings led us to examine the interplay between C9ORF72 expression, and regulation of autophagy in rodent and human models of C9ORF72-ALS.



III) Elucidating the timing of TDP-43 related phenotypes using iPSC-derived motor neurons from TARDBP ALS patients

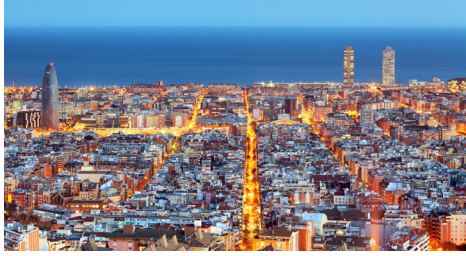
Melissa Nijs*(1)(2), Adria Sicart Casellas (1)(2), Jimmy Beckers (1)(2), Raheem Fazal (1)(2), Ludo Van Den Bosch (1)(2), Philip Van Damme (1)(2)(3)

1 KU Leuven - University of Leuven, Department of Neurosciences, Experimental Neurology and Leuven Brain Institute (LBI), Leuven, Belgium.

2 VIB - Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium.

3 University Hospital Leuven, Department of Neurology, Leuven, Belgium.

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal adult-onset neurodegenerative disease, characterized by the selective loss of both upper and lower motor neurons. In 3-7% of familial patients, the disease is caused by inherited mutations in the Transactive response DNA binding protein (TARDBP) gene, encoding the TDP-43 protein. However, cytoplasmic hyperphosphorylated and ubiquitinylated TDP-43 protein aggregates are found in neurons and glial cells of 97% of ALS patients, including in patients with a hexanucleotide repeat expansion in Chromosome 9 open reading frame 72 (C9orf72) and all sporadic cases. Therefore, TDP-43 pathology is considered as a pathological hallmark of ALS. Our laboratory has previously shown that iPSC-derived spinal motor neurons (sMNs) harboring a heterozygous mutation in the TARDBP gene also display TDP-43 pathology with cytoplasmic mislocalization, C-terminal cleavage and accumulation of insoluble TDP-43. Additionally, axonal transport deficits have been observed. In this study, we now aim to elucidate the timing and order in which each of these phenotypes occur. To this end, live cell imaging, immunocytochemistry and western blot analysis are performed on TARDBP mutant sMNs, as well as their isogenic controls, at multiple timepoints during sMN differentiation. Preliminary results indicate that mitochondrial axonal transport is already impaired at the first timepoint, whereas changes in TDP-43 solubility and C-terminal cleavage are only observed at a later stage. These findings suggest that deficits in axonal transport might precede the accumulation of insoluble TDP-43 and C-terminal cleavage fragments.



IV) Dynamic Translational Profile of Stressed iPSC-MNs from C9orf72-ALS Patients by Translating Ribosome Affinity Purification (TRAP)

Yinyan Xu* (1,2), Chaitra Sathyaprakash (1), Krisya Louie (1), Ruxandra Dafinca (1), Jakub Scaber (1), Kevin Talbot (1)

1. Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom.

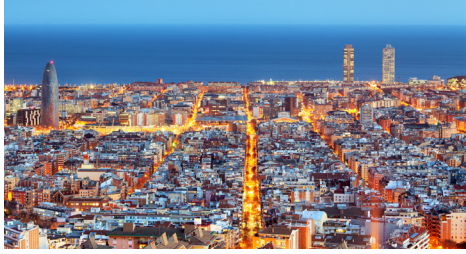
2. Chinese Academy of Medical Sciences (CAMS), CAMS Oxford Institute (COI), Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

Background: The G4C2 Hexanucleotide Repeat Expansion (HRE) in the gene C9orf72 is the commonest genetic cause of ALS. The intronic repeat RNA accumulates intracellularly as RNA foci and is translated non-canonically into dipeptide repeat proteins (DPRs), both of which have been shown to affect RNA metabolism. Furthermore, impaired global translation and perturbed stress granule dynamics have been associated with C9orf72-ALS. We hypothesize that the C9orf72 HRE may lead to a difference in the profile of translating mRNAs (translatome) in motor neurons at baseline, after stress or during recovery.

Methods: In this study, we treated human induced Pluripotent Stem Cell-derived Motor Neurons (iPSC-MNs) from 3 healthy controls (HCs) and 3 C9orf72-ALS patients with 0.5 mM sodium arsenite (ARS) for one hour, and obtained the transcriptome and translatome at baseline, immediately after stress, and after 2 h of recovery. To validate the findings from RNA sequencing, we characterized the ARS-induced stress response for up to 24 h after stress removal, through a range of biochemical experiments.

Results: We found a similar transcriptomic profile in C9orf72-ALS iPSC-MNs compared to HCs after stress. However, a discrete group of 68 Differentially Expressed Genes (DEGs) were identified in the translatome of C9orf72-ALS iPSC-MNs after 2 h of recovery. Notable DEGs relevant to ALS pathogenesis include UNC13A and PURA, and GO term analysis of the DEGs shows enrichment in synaptic function and neuronal projection. Gene Set Enrichment Analysis (GSEA) suggests an upregulation of antioxidant activity, apoptosis, translation and ribosome biogenesis, and a downregulation of synapse organization, axonal development and cellular signalling in C9orf72-ALS iPSC-MNs during early recovery. However, we did not detect an abnormal stress response in the disease lines in terms of cell viability, immunoblotting of apoptotic, autophagic and global translation activity markers, and stress granule dynamics. We also examined C9orf72 protein expression and the nuclear/cytoplasmic ratio of TDP-43 at different time points after stress and did not see a significant difference between C9orf72-ALS and HC.

Conclusions and plans: The translatome of C9orf72-ALS iPSC-MNs reveals a number of dysregulated genes and pathways during early recovery from transient ARS treatment. Future experiments will investigate the spatial association of selected DEG mRNAs with C9orf72 HRE foci and stress granule prote



V) MAM lipidome changes associated with TDP-43 dysfunction

Anna Fernàndez-Bernal*(1), Meritxell Martin Gari(1), Natalia Mota-Martorell (1), Pol Andrés-Benito (2), Mònica Povedano (3), Elia Obis (1), Isidro Ferrer (3), Reinald Pamplona (1), Manuel Portero-Otin (1)

(1) Metabolic Pathophysiology Research Group, Department of Experimental Medicine, University of Lleida-IRB Lleida, Lleida, Spain.

(2) University of Barcelona-CIBERNED, L'Hospitalet de Llobregat, Barcelona, Spain.

(3) Neurology Service, Bellvitge University Hospital, L'Hospitalet de Llobregat, Barcelona, Spain

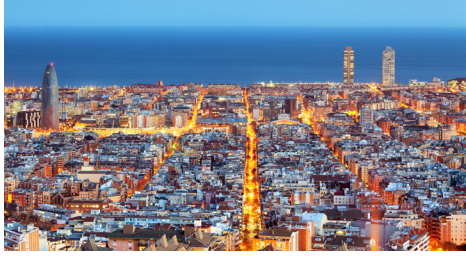
INTRODUCTION: TDP-43 may contribute to ALS pathogenesis in different cellular compartments like mitochondria. Mitochondria show intimate contact with the particular endoplasmic reticulum (ER) membrane subdomains termed mitochondrial-associated membranes (MAMs). MAM lipid composition is essential for its proper function. Consequently, changes in their lipidome could compromise the activity of proteins residing in MAMs. Our previous finding that TDP-43 alterations in animal and cellular models are related to changes in MAM activity led us to assess the possible relationship between TDP-43 (dys)function and MAM lipidome.

OBJECTIVE: To evaluate the potential changes in MAM lipidome secondary to TDP-43 alterations and establish a relationship between TDP-43 and lipid metabolism.

METHOD: We evaluated the lipidome of MAM and ER in the human frontal cortex (n=5 control group; n=6 ALS group) and tissue from transgenic B6N-Cg-Tg(Prnp-TARDBP*Q331K)103Dwc/J (TDP-43 Q331K) mice, both in brain and spinal cord samples. After subcellular fractionation, we used liquid chromatography-mass spectrometry (LC-MS) to perform an untargeted lipidomic approach. To assess the potential alterations in MAM-controlled lipid metabolism related to TDP-43 dysfunction, we have also evaluated phospholipid metabolism in a human cellular model of TDP-43 loss of function.

RESULTS: Untargeted lipidomics analysis shows that MAM and ER lipid composition is different in humans and animals. In addition, we also observed that alterations in TDP-43 affect part of MAM and ER lipidome in all of the analyzed samples. In contrast, the decrease of TDP-43 levels in HeLa-PLKO cells does not lead to global changes in phospholipid metabolism, suggesting that extra-MAM lipid metabolism (Kennedy pathway), could compensate for potential loss of TDP-43 controlled pathways.

CONCLUSION: Abnormalities in TDP-43 can affect the lipid composition of MAM and ER, both in human and animal models of mutated TDP-43. Changes in MAM composition may affect proteins that reside in them, which would partly explain previous results in which we have observed alterations in MAM activity in models of TDP-43 dysfunction.



VI) Gap junctions are functionally enhanced in iAstrocytes derived from C9ORF72 repeat expansion patients

Iris S Pasniceanu*, Jannigje Kok, Allan C Shaw, Cleide Dos Santos Souza, Pamela J Shaw, Laura Ferraioulo, Matthew R Livesey

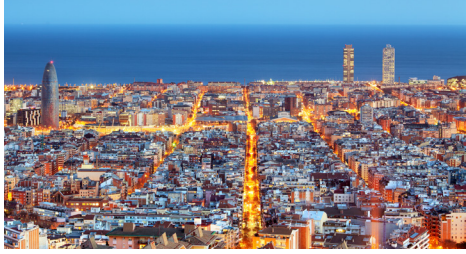
Sheffield Institute for Translational Neuroscience, Department of Neuroscience University of Sheffield, United Kingdom

INTRODUCTION: ALS is an incurable neurodegenerative disease, in which the C9ORF72 repeat expansion (C9RE) is a major causal genetic impairment. The same mutation gives rise to FTD, placing the C9RE at the centre of an ALS-FTD spectrum. Astrocytes are important non-neuronal cell types that provide functional and structural support to neurons but become toxic in ALS-FTD. In vitro, astrocytes exhibit toxicity through intercellular communication and secreted factors, thus indicating a source of dysfunction at the astrocyte membrane. The mechanisms of astrocyte toxicity and dysfunction remain to be fully understood. Here we have electrophysiologically investigated membrane dysfunction in C9RE astrocytes.

METHODS: Enriched in vitro cultures of S100δ+ astrocytes were generated from fibroblasts using a direct induction protocol (iAstrocytes). Fibroblasts were obtained from healthy subjects and patients living with C9RE. Whole-cell patch-clamp electrophysiology was used to characterise iAstrocyte membrane function.

RESULTS: Intrinsic astrocyte membrane currents were evoked using a voltage-step protocol and current-voltage relationships established for each iAstrocyte line. For C9RE iAstrocytes, the data showed a significant gain-of-function enhancement in passive current amplitudes versus control iAstrocytes (3-fold increase in C9RE compared to controls; $p<0.0001$, Two-way ANOVA with Tukey's tests). Importantly, the differences in intrinsic membrane properties indicate that the observations are not due to accelerated maturation (rectification index of passive currents shows no consistent differences between C9 and controls, $p\geq0.08$), but an increase in the expression of a specific ion channel/transporter in C9RE iAstrocytes. To explore this we pharmacologically characterised the evoked membrane currents of each line. Using selective pharmacology we have determined that the gain-of-function in C9RE iAstrocytes is directly associated with the up-regulation of gap junctions (10µM CBX significantly decrease current amplitude, $p<0.0001$, Two-way ANOVA with Tukey's tests).

CONCLUSIONS: C9RE iAstrocytes display gain-of-function membrane current enhancement due to an upregulation of gap junction membrane channels. Gap junction expression is directly associated with the transport of molecules that impact on neuronal viability. Our work will now investigate the impact of C9RE iAstrocytes on the viability of neurons and the mechanisms giving rise to these alterations.



VII) Targeting De Novo Fatty Acid Synthesis as a Therapeutic Strategy to Alleviate Non-cell Autonomous Mechanisms in ALS

Maddi Garciandia-Arcelus (1)*, Andrés Jiménez (1,2), Laura Rodríguez (1), Gorka Gereñu (1,2,3), Jon Ondaro-Ezkurra (1,2), José Ignacio Ruíz-Sanz (3), Roberto Fernández-Torrón (1,4), Juan José Poza-Aldea (4), Javier Riancho (2,5), Raul Domínguez (6), Juan Bautista-Espinal (4), Jesus María Aizpurua (7), Gonzalo González-Chinchón (8,9), Miren Zulaica (1,2), María Begoña Ruiz-Larrea (3), Mónica Povedano (6), Adolfo López de Munain (1,2,4,9,10), Gorka Fernández-Eulate (1,4,11,12), Francisco Javier Gil-Bea (1,2,13,14).

(1) Neuroscience Area, Biodonostia Health Research Institute, Donostia-San Sebastián, Spain.

(2) CIBERNED, ISCIII (CIBER, Carlos III Institute, Spanish Ministry of Sciences and Innovation), Madrid, Spain.

(3) Department of Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain.

(4) Department of Neurology, Donostialdea Integrated Health Organization, Osakidetza Basque Health Service, Donostia-San Sebastián, Spain.

(5) Department of Neurology, Sierrallana-IDIVAL Hospital, Torrelavega, Spain.

(6) Department of Neurology, Bellvitge University Hospital, Barcelona, Spain.

(7) Department of Organic Chemistry, Faculty of Chemistry, University of the Basque Country (UPV/EHU), Donostia-San Sebastián, Spain.

(8) Department of Neurology, Araba University Hospital, Osakidetza Basque Health Service, Vitoria-Gasteiz, Spain.

(9) Department of Neurosciences, University of the Basque Country (UPV/EHU), Donostia-San Sebastián, Spain.

(10) Faculty of Medicine, University of Deusto, Bilbao, Spain.

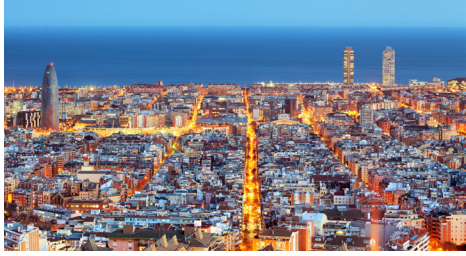
(11) Institut Necker-Enfants Malades, INSERM U1151, BioSPC (ED562), Université Paris Cité, Paris, France.

(12) Nord/Est/Ile-de-France Neuromuscular Reference Center, Institut de Myologie, Pitié-Salpêtrière Hospital, Paris, France.

(13) Ikerbasque Basque Foundation for Science, Bilbao, Spain.

(14) Department of Health Sciences, Public University of Navarra (UPNA), Pamplona, Spain.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive degenerative motor neuron disease with no effective treatment. Exploration of lipid metabolism has led to the identification of descriptive biomarkers of disease, providing insights into the underlying biological processes involved in a diverse range of psychiatric and neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Clinical and experimental studies have also highlighted the significance of lipids in the neurodegenerative process of ALS. In fact, several lipid-lowering approaches have been tested in the disease, showing promising results. Our findings reveal that the progression of ALS is associated with a dysregulated elongation of long-chain fatty acids in serum samples from two distinct follow-up patient cohorts. Lipotoxicity induced by glial cells via elongation of saturated fatty acids has been recently identified as a potential mediator of neurodegeneration, and inhibiting this process has been proposed as a neuroprotective therapy. Our research indicates that TDP-43 or FUS loss-of-function in both muscle and glial cells, is implicated in the dysregulation of elongation of saturated fatty acids. In addition, inhibition of long-chain fatty acid elongation, achieved through both genetic and pharmacological means using a potent, selective, and orally bioavailable inhibitor, consistently leads to improved neuromuscular phenotypes and increased life expectancy in fruit flies with muscle-specific TDP-43 deficiency. To summarize, we propose that therapeutic interventions aimed at reducing the accumulation of saturated fatty acids by inhibiting their de novo synthesis, could alleviate the non-cell autonomous degenerative mechanisms that supportive cells exert on vulnerable motor neurons in ALS.



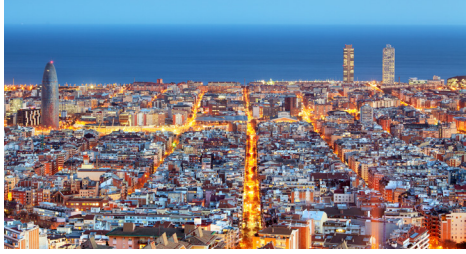
VIII) Epigenetic analysis on organoids for the study of Amyotrophic Lateral Sclerosis

Evelyn Scarian (1), Matteo Bordoni (1), Camilla Viola (2), Francesca Dragoni (1,2), Rosalinda Di Gerlando (1,2), Luca Diamanti (1), Stella Gagliardi (1), Orietta Pansarasa (1)

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease (NDD) with a progressive clinical curse which affects upper and lower motor neurons (MNs), causing weakness, muscle atrophy and spasticity. As for other NDDs there are two typical forms of ALS, the sporadic (sALS), which account for 90% of the cases, and the familial one. Until now, only symptomatic treatments are available, especially for the lack of realistic models which can mimic the physiological environment in which cells grow. One important innovation are organoids. Organoids are pluripotent stem cell-derived self-organizing structures which can recapitulate the tissue of origin in vitro. Brain organoids contain both neural and glial cells and are used for disease modeling, i.e. for the study of cells interactions and of neurodevelopment.

In our laboratory, we already optimized a protocol for motor neuron organoids (MNOs) formation from CTRL and sALS NSCs. Aim of this project was the investigation of the epigenetic characteristics of organoids at each differentiation step, neural stem cells organoids (NSCO), motor neuron progenitors organoids (MNOPs) and MNOs and the comparison of these characteristics which the ones of 2D cultured cells. We performed an ELISA assay for DNA methylation status on 5-methylcytosine (5-mC) finding a decreased methylation ($p^* < 0.05$) in sALS MNOs when compared to 2D MNs. Moreover, we performed western blot analysis to test the expression of two key methylating enzymes, the DNA methyltransferase 1 (Dnmt1) and the DNA methyltransferase 3a (Dnmt3a). We found a significant decrease in the protein expression of Dnmt1 in both CTRL and sALS MNPOs and MNOs when compared to the corresponding NSCOs, whereas we did not find any significant difference in Dnmt3a expression. Moreover, we are evaluating the gene expression of Dnmt1 and Dnmt3a by RT-qPCR and we found some differences both between organoids and 2D cultured cells and between CTRL and sALS cultures. Finally, we performed western blot analysis to test the methylation status of two sites involved in ALS pathology, lysine 9 and lysine 27, both on histone 3, finding an increasing trend of their protein expression during the differentiation protocol.

In conclusion, we confirmed the major deregulation of MNOs when compared to 2D cultured cells, already seen by our group through RNA-seq, and the possibility to use organoids as a useful tool for the study of epigenetics in ALS.



IX) Cognate microglia – T cell interaction induce neurotoxic T cell function in a fast-progressing C9orf72 ALS animal model

Chao Yang (1), Elisabeth Scharf (1), Steffanie Heindl (2), Leonard Lala (1), Stefan Roth (2, 3), Meike Michaelsen (1), Thomas Arzberger (1, 3, 4, 5), Arthur Liesz (2, 3), Dieter Edbauer (1, 3, 6), Qihui Zhou (1,3) (*)

(1) German Center for Neurodegenerative Diseases, Munich, Germany.

(2) Institute for Stroke and Dementia Research, University Hospital, Ludwig Maximilians University Munich, Munich, Germany.

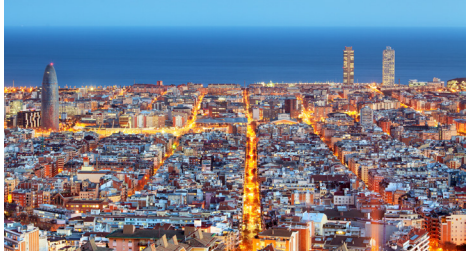
(3) Munich Cluster for Systems Neurology (SyNergy), 80336, Munich, Germany.

(4) Center for Neuropathology and Prion Research, Ludwig-Maximilians-University Munich, 81377, Munich, Germany.

(5) Department of Psychiatry and Psychotherapy, University Hospital, Ludwig-Maximilians-University Munich, 80336, Munich, Germany.

(6) Graduate School of Systemic Neurosciences (GSN), Ludwig-Maximilians-University Munich, 81377, Munich, Germany.

Protein aggregation and neuroinflammation are the two hallmarks of neurodegenerative diseases. ALS and FTD are progressive fatal neurodegenerative diseases with overlapping clinical symptoms and neuropathological findings. The hexanucleotide repeat expansion (G4C2)n in the C9orf72 locus is the most common genetic cause of ALS/FTD and has two main pathological consequences: a loss-of-function effect causing C9orf72 haploinsufficiency and toxic gain of function due to bidirectional transcription of (G4C2)n repeat. In the CNS of a fast-progressing C9orf72 animal model, congenic expression of poly-GA in neurons elevated microgliosis and pro-inflammatory interferon responses. We noticed a robust accumulation of CD3+ T cells during disease progression in the brain of C9orf72 transgenic mice. Cognate microglia – T cell interaction was suggested by histology and flow cytometry. Furthermore, whole-body depletion of CD3+ T cells resulted in higher body weight, prolonged life expectancy, and reduced behavior deficits, suggesting a neurotoxic function of accumulated CD3+ T cells in the brain of C9orf72 transgenic mice. Collectively, our data suggest a neurotoxic interaction of microglia with CD3+ T cells in C9orf72 ALS/FTD.



X) An interaction between synapsin and C9orf72 regulates excitatory synapses and is impaired in ALS/FTD

Claudia S. Bauer (1) (*), Rebecca N. Cohen (1), Francesca Sironi (2), Matthew R. Livesey (1), Thomas H. Gillingwater (3, 4), J. Robin Highley (1), Daniel J. Fillingham (1), Ian Coldicott (1), Emma F. Smith (1), Yolanda B. Gibson (1), Christopher P. Webster (1), Andrew J. Grierson (1), Caterina Bendotti (2), and Kurt J. De Vos (1).

1 Sheffield Institute for Translational Neuroscience (SITraN), Department of Neuroscience, University of Sheffield, Sheffield, UK.

2 Laboratory of Molecular Neurobiology, Department of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy.

3 Edinburgh Medical School: Biomedical Sciences, University of Edinburgh, Hugh Robson Building, Edinburgh, UK.

4 Euan MacDonald Centre for Motor Neuron Disease Research, Chancellor's Building, University of Edinburgh, Edinburgh, UK.

Introduction

Synaptic dysfunction and degeneration of synapses are pathophysiological hallmarks of neurodegenerative diseases, including amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). The main genetic cause of ALS/FTD is a GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene (C9ALS/FTD) which leads to reduced expression of the C9orf72 protein. How C9orf72 protein haploinsufficiency impacts on neuronal function and contributes to C9ALS/FTD pathology is still unknown.

Here we reveal that C9orf72 plays a novel cell-autonomous role in the regulation of synaptic vesicle pools and neurotransmission at excitatory synapses. Our data propose that C9orf72 haploinsufficiency is a major contributor to synaptic dysfunction in C9ALS/FTD.

Methods

We investigated the role of C9orf72 at the synapse in vitro and in vivo using molecular biology, protein biochemistry, high resolution fluorescence and electron microscopy as well as electrophysiology techniques.

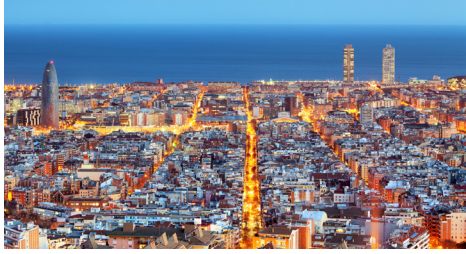
Results

We identified the synapsin protein family as new endogenous interactors of C9orf72 at synapses and mapped the interaction to the N-terminal longin domain of C9orf72 and the conserved C-domain of synapsin. Synapsins are the most abundant family of synaptic vesicle proteins which modulate neurotransmission by regulating synaptic vesicle pools.

Functionally, C9orf72 deficiency reduced the number of excitatory synapses and decreased synapsin levels at remaining synapses in vitro in hippocampal neuron cultures. Similarly, synapses were reduced in a gene-dosage-dependent manner in vivo in the hippocampal mossy fibre system of heterozygous and homozygous C9orf72 knockout mice. Consistent with synaptic dysfunction, electrophysiological recordings in hippocampal neuron cultures with reduced C9orf72 expression revealed impaired excitatory neurotransmission and network function, which correlated with a severe depletion of synaptic vesicles from excitatory synapses in the hippocampus of C9orf72 knockout mice. Finally, neuropathological analysis of post-mortem sections of C9ALS/FTD patient hippocampus with C9orf72 haploinsufficiency revealed a marked reduction in synapsin.

Conclusions

Thus, our results indicate that disruption of the interaction between C9orf72 and synapsin may contribute to ALS/FTD pathobiology.



XI) Cortical network dysfunction in ALS using task-free magnetoencephalography

Michael Trubshaw(1,2)*, Chetan Gohil(1), Katie Yoganathan(1,2), Evan Edmond(1,2), Malcolm Proudfoot(1,2), Mark Woolrich(1), Martin R. Turner(2)

1. Wellcome Centre for Integrative Neuroimaging, University of Oxford, Oxford, United Kingdom
2. Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

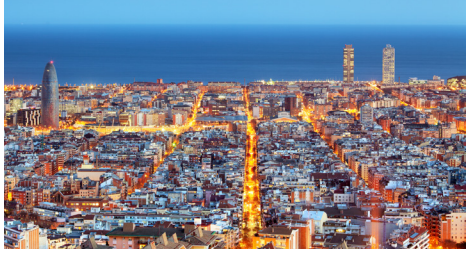
ALS involves complex primary pathological and compensatory dysfunction of cerebral networks. Callosal (inter-hemispheric pathway) pathology has been a consistent observation in histological and MRI studies in ALS. Magnetoencephalography (MEG) is a brain imaging technique which non-invasively measures the micro-magnetic fields generated by oscillatory brain activity. Modern computational techniques allow analysis of this data to provide highly temporally and spatially localised information about cortical neurophysiology. Task-free, resting-state MEG offers unique insight into cerebral pathophysiology by revealing dynamic, network-level changes in oscillatory brain power and connectivity.

Thirty-six non-demented patients with apparently sporadic ALS and 51 age- and gender-matched controls underwent an 8-minute resting-state MEG recording and structural MRI scan. We calculated oscillatory cortical power, connectivity and complexity measures in 52 regions and 5 canonical frequency bands (δ, δ, δ, δ, δ). We used maximum statistic permutations tests to look for differences between groups, and the effect of disability (ALSFRS) on brain function, correcting for age, gender and handedness.

ALS patients showed decreased beta power in sensorimotor regions (p=0.034) and increased gamma power in frontotemporal regions (p=0.015). Frontotemporal activity complexity was also increased in ALS patients (p=0.008). Higher levels of ALS patient disability were associated with increased power in these same regions (p=0.007) and increased frontotemporal connectivity overall (p=0.005) but relatively more with the contralateral hemisphere (p=0.034).

A fall and regional shift of sensorimotor beta power is the consistent underpinning of ALS cerebral pathophysiology. Increased power, connectivity and complexity of brain activity in frontotemporal regions may reflect a distinct process within ALS and FTD's pathological spectrum, or compensatory response to primary motor system disintegration. Altered functional laterality may reflect specific damage to inter-hemispheric fibres of the corpus callosum. MEG may offer a biomarker for disease progression in ALS and secondary outcome measure in therapeutic trials. The extent to which MEG network changes occur pre-symptomatically requires dedicated exploration in carriers of pathological genetic variants.

Session 7. Epidemiology

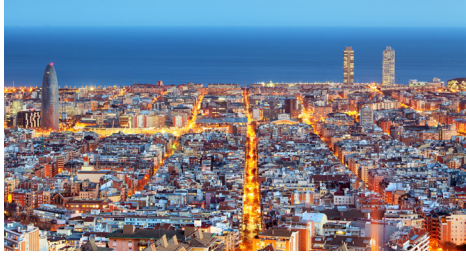


28. Genetic and Environmental Risk Factors contribute in the pathogenesis of ALS in Cyprus

Ellie Mitsi (1)(*), Kyproula Christodoulou (1), Christiana Christodoulou (2), Pantelitsa Koutsou (1), Anthi Georghiou (1), Eleni Papanicolaou-Zamba (2), Paschalis Nicolaou (1)

(1) Neurogenetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.
(2) Neuroepidemiology Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons, presenting with relentlessly progressive muscle atrophy and weakness. Since the identification of the first causative gene SOD1 in the 1990s and with recent advances in genetics, more than 50 potential causative or disease-modifying genes have been identified, with SOD1, TARDBP, FUS and C9orf72 being the commonest. However, the etiology of ALS remains unexplained for over 85% of all cases, suggesting that various environmental factors are implicated in the pathogenesis of the disease. This study aimed to conduct a detailed genetic epidemiological investigation and to detect potential exogenous risk factors of ALS in the Cypriot population.A total of 82 ALS patients provided the cohort for the variant screening in the genes C9of72, SOD1, TARDBP, FUS, ATXN2, and SMN1 that have been previously found to be associated with ALS. In addition, a case-control study was conducted with a total of 56 ALS patients and 56 healthy controls, to investigate the contribution of known environmental risk factors with disease. Demographic, lifestyle characteristics and environmental risk factors were collected through the use of a detailed questionnaire. Results revealed one patient with the pathogenic c.800A>G (p.Asn267Ser) genetic variant in the TARDBP (1.25%) and 16 additional patients with a pathogenic hexanucleotide repeat expansion in C9orf72 (20%) have been found. No pathogenic variants have been identified in the remaining genes. Furthermore, statistical analysis of the case-control study revealed a statistically significance (p=0.000461) in smoking between the two groups. Univariate logistic regression analysis was performed for the exogenous risk factors including exposures to chemicals, head trauma and electric injury. Statistically significant results were identified for head trauma/injury (p=0.035), electric injury (p=0.0066) and exposure to chemicals (p=0.0015). Collectively, findings indicate that C9orf72 repeat expansions are indeed causative for ALS in the Cypriot population, and agree with findings from other European countries. However, genetic clusters of the pathogenic variants in the remaining genes are not present in Cyprus. Finally, the case-control investigation has shed some light on the epidemiological data of ALS in Cyprus, by indicating various environmental determinants of ALS in the Cypriot population.



29. Incidence, mortality and survival of patients with amyotrophic lateral sclerosis (ALS) in Belgrade, Serbia (1994-2018)

Aleksa Palibrk (1), Ivo Bozovic (1), Vukan Ivanovic (1), Stojan Peric (1), Ivana Basta (1), Vladimir Nikolic (2), Ivan Soldatovic (3), Zorica Stevic (1)

1 Neurology Clinic, University Clinical Center of Serbia, Belgrade, Serbia.

2 Institute of Epidemiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

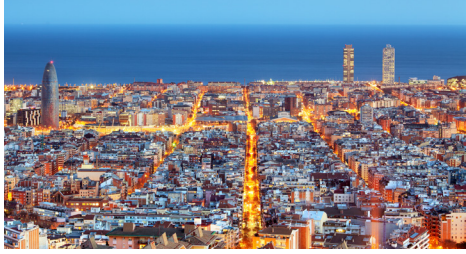
3 Institute of Medical Statistics and Informatics, University of Belgrade, Belgrade, Serbia.

Introduction: Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease which affects both upper and lower motor neurons. Various studies showed an increase in incidence over the last two decades. There were no epidemiological studies for ALS patients in Serbia during this period. The aim of our study was to determine incidence and mortality rates in Belgrade, Serbia, during 24 year period (1994-2018), as well as to determine risk factors for worse survival.

Methods: Our study included 557 patients with ALS who were diagnosed and treated at Neurology Clinic, University Clinical Center of Serbia between 1994 and 2018. Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) was used. Standardized incidence rates per 100,000 inhabitants were calculated by direct standardization method using World Standard Population., as well as mortality rates.

Results: Out of 557 ALS patients, 325 (58.3%) were male. Average age at diagnosis was 61.3±10.9 years. Spinal onset was noted in 423 (75.9%) of our patients. Average ALSFRS-R at the time of diagnosis was 39.4±6.9. Median survival was 36 months. Significant difference regarding the survival was noted in age groups (<60: 48 (43.4-52.6); 60+: 36 (35.2-36.8), p=0.0007), form (spinal 42.0 (39.6-44.4); bulbar 36.0 (32.9-37.6) p<0.001), ALSFRS-R (<38: 36 (32.7-39.3); 38+: 42 (39.4-44.6) p=0.013). Overall 3, 5 and 10 year survival was 49.8%, 26.2% and 7.1% respectively. In age<60 years group, 3, 5 and 10 year survival was 57.3%, 33.0% and 10.4% while in age 60+ group was 44.7%, 21.3% and 4.7%, respectively. Crude incidence rate was between 1.17 (1994-1998)-2.45 (2014-2018)/100000, while five-year age-standardized incidence rate was between 0.57-1.05/100000. Incidence rates significantly increased during the period of 24 years with positive linear trend of 0.04 increase by year (p=0.001). Crude mortality rate was between 4.1 (2014-2018)-9.7 (1999-2003)/100000 with no obvious trend of change during the study. Both incidence and mortality rates increased with age. In all age groups, incidence was significantly higher in men.

Conclusion: Our study observed increase of incidence of ALS patients in Belgrade, Serbia, during 24 years, with positive linear trend of 0.04 increase by year. Older age at diagnosis, lower ALSFRS-R at the time of diagnosis and bulbar onset were recognized as significant factors for worse survival.



30. Presymptomatic geographical distribution of ALS patients suggests the involvement of environmental factors in the disease pathogenesis

Rosario Vasta(1)*, Stefano Callegaro(1), Silvia Sgambetterra(2), Sara Cabras(1,3), Francesca Di Pede(1), Filippo De Mattei(1), Enrico Matteoni(1), Maurizio Grassano(1), Alessandro Bombaci(1), Giovanni De Marco(1), Giuseppe Fuda(1), Giulia Marchese(1), Francesca Palumbo(1), Antonio Canosa(1,4,5), Letizia Mazzini(6), Fabiola De Marchi(6), Cristina Moglia(1,4), Umberto Manera(1,4), Adriano Chiò(1,4,5), Andrea Calvo(1,4)

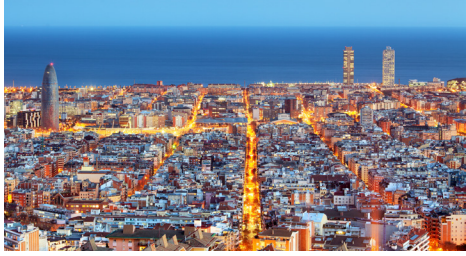
(1)ALS Center, Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy.
(2) Department of Neuroscience "Rita Levi Montalcini", University of Turin, Turin, Italy.
(3)International School of Advanced Studies, University of Camerino, Camerino, Italy.
(4) Neurology 1, AOU Città della Salute e della Scienza di Torino, Turin, Italy.
(5) Institute of Cognitive Science and Technologies, National Research Council, Rome, Italy.
(6) ALS Center, Department of Neurology, Azienda Ospedaliero Universitaria Maggiore della Carità, and University of Piemonte Orientale, Novara, Italy.

Background. Given that the pathogenetic process of ALS begins many years prior to its clinical onset, examining patients' residential histories may offer insights on the disease risk factors. Here, we analyzed the spatial distribution of a large ALS cohort in the 50 years preceding the disease onset.

Methods. Data from the PARALS register was used. A spatial cluster analysis was performed at the time of disease onset and at 1-year intervals up to 50 years prior to that.

Results. A total of 1124 patients were included. The analysis revealed a higher-incidence cluster in a large area (435000 inhabitants) west of Turin. From 9 to 2 years before their onset, 105 cases were expected and 150 were observed, resulting in a relative risk of 1.49 ($p = 0.04$). We also found a surprising high number of patients pairs (51) and trios (3) who lived in the same dwelling while not being related. Noticeably, these occurrences were not observed in large dwellings as we would have expected. The probability of this occurring in smaller buildings only by chance was very low ($P=0.01$ and $P=0.04$ for pairs and trios, respectively).

Conclusions. We identified a higher-incidence ALS cluster in the years preceding the disease onset. The cluster area being densely populated, many exposures could have contributed to the high incidence ALS cluster, while we could not find a shared exposure among the dwellings where multiple patients had lived. However, these findings support that exogenous factors are likely involved in the ALS pathogenesis.



31. Epidemiological Trends of Amyotrophic Lateral Sclerosis (ALS) in Ireland 1996-2021

Robert McFarlane(1)*, Mark Heverin(1), Cathal Walsh(1), Orla Hardiman(1)

Academic Unit of Neurology, Trinity College Dublin

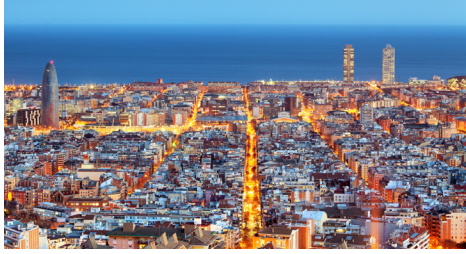
Objectives: To examine the incidence, prevalence, age of onset and survival of patients diagnosed with Amyotrophic Lateral Sclerosis (ALS) in the Republic of Ireland over 25 years.

Methods: Incident and prevalent cases of ALS were estimated using the Irish population based ALS Register, which has been continuous operation since 1994. Incident cases were age standardised using the direct method and applied to three standard populations (Irish, European, and American). Survival was determined using Kaplan-Meier curves and Cox regression models. Non-normally distributed groups were compared using the Kruskal-Wallis test with a Bonferroni correction.

Results: 2771 patients with ALS were identified in the Republic of Ireland over 25 years. Incidence per 100,000 was determined for the population over the age of 15 years. Crude incidence increased from 2.64 to 5.46 per 100,000 . Standardised incidence increased from 2.64) to 3.1) per 100,000. Prevalence increased from 5.83 to 8.10 per 100,000. The median age of onset increased from 64 to 67 . The peak age of incidence increased from those between 70-74 to those between 75-79 . Overall, women had a consistently later median age of onset of 67 compared to men at 65 ($p<0.001$). No difference in survival was noted.

Older age at onset (HR1.02 CI1.02-1.03), bulbar onset disease (HR1.25 CI1.15-1.37), respiratory onset disease (HR1.57 CI1.19-2.08) were negative predictive factors in multivariate Cox regression analysis. Riluzole use (HR0.85 CI0.77-0.93), multidisciplinary clinic attendance (HR0.76 CI0.68-0.84) and diagnostic delay (HR0.99 CI0.99-0.99) were positive predictive factors.

Conclusions: Within the Republic of Ireland, age-standardised overall incidence, peak incidence, prevalence, and age of onset of ALS increased over 25 years. Despite widespread use of NIV, aggressive secretion management, and changes in ALS care; mean survival has not changed.



32. Population-level penetrance of ALS genes is markedly reduced

Andrew G. L. Douglas* (1, 2), Diana Baralle (2, 3)

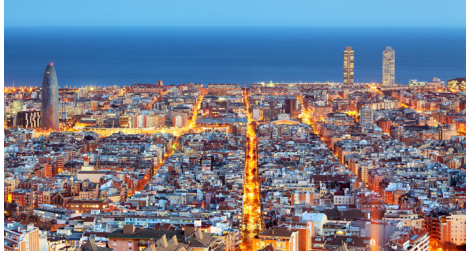
1. Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK.
2. Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK.
3. Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, UK.

Background: Amyotrophic lateral sclerosis (ALS) has a lifetime risk of around 1 in 400 people and 5-10% of cases are reported to be familial. Known predisposition genes account for 60-70% of familial cases. However, pathogenic or likely pathogenic gene variants have been found in 11-21% of all ALS cases, most of which appear to be sporadic, indicating substantially reduced gene penetrance. In this study we have quantified the prevalence of disease-causing mutations in a general population database and have thereby estimated the population-level penetrance of ALS genes.

Methods: The expected population frequencies of mutations in known ALS genes, assuming full penetrance, were calculated using figures from the published literature. The numbers of pathogenic and likely pathogenic variants in ALS genes (as annotated in the ClinVar database) was ascertained by manual examination of the gnomAD database as a surrogate for the general population.

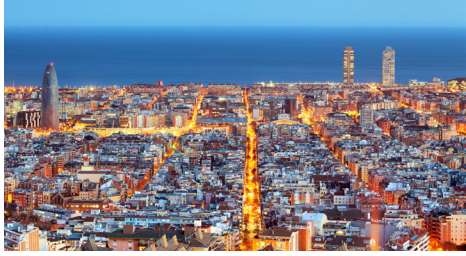
Results: The number of SOD1 pathogenic or likely pathogenic variants was found to be 5.4-fold higher than expected, indicating a population-level penetrance of only 18%. TARDBP and FUS variants were similarly found to have 5.9-fold and 7.4-fold higher prevalence than expected, indicating a penetrance of 17% and 14% respectively.

Conclusion: The population-level penetrance of ALS disease-causing gene variants is markedly reduced. These results are in keeping with the clinical experience of such gene variants being identified in apparently sporadic ALS cases. The reduced penetrance of ALS genes in the general population has significant implications for the genetic counselling of unaffected relatives of patients found to have such gene variants. Given that pre-symptomatic therapeutic trials of intrathecal antisense oligonucleotide treatment are currently under way for carriers of SOD1 variants, this finding also has major implications for the decision of whether or not to treat unaffected individuals carrying such variants.



33. Epidemiology studies in Colombia
Martha Peña Preciado

Session 8. Applied Neurophysiology



34. Acceptability and feasibility of the MiNDToolkit intervention for management of behavioural symptoms in MND: the views of healthcare professionals

*Thando Katangwe-Chigamba (1), Emma Flanagan (1), Polly-Anna Ashford (1), Eneida Mioshi (2)

(1) Norwich Clinical Trials Unit, Norwich Medical School, University of East Anglia, UK.
(2) School of Health Sciences, University of East Anglia, UK.

Background

There is increasing evidence that people with Motor Neurone Disease (MND) can display complex changes in behaviour, personality, and cognitive functioning, with or without, Frontotemporal Dementia. Changes in behaviour are present in at least 50% of patients with MND (50-75%, depending on the clinical assessment used), where 15% of patients present with MND with frontotemporal dementia (MNDFTD).

The practical burden and care challenges that these non-motor impairments present to both carers and healthcare professionals calls for the development of interventions that might help to manage these symptoms. Working with multiple stakeholders including carers, healthcare professionals (HCPs) and expert clinicians we have co-created and feasibility tested the MiNDToolkit online intervention for the management of behavioural impairment in MND. The MiNDToolkit is a novel psychoeducational intervention delivered to carers by a bespoke online platform, and the intervention is reinforced by trained healthcare professionals.

Aim

In a qualitative process sub-study, we sought to generate an understanding of intervention implementation across specialist MND teams in England and Wales and explore the acceptability of the intervention from the perspective of HCPs.

Methods

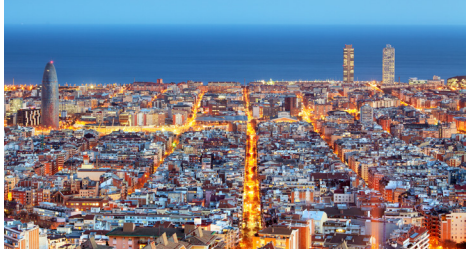
We conducted ten semi-structured interviews with HCPs purposively sampled for their role and engagement with the MiNDToolkit intervention. A framework approach, complemented with Medical Research Council process evaluation guidance and comparison of emerging themes, was used to analyse transcribed data.

Results

The MiNDToolkit training and platform were acceptable, with educational information and suggested techniques increasing HCP knowledge and confidence with the management of behavioural symptoms. HCP engagement with the platform and reinforcement of techniques with carers was variable, and largely dependent on capacity and whether consultations were home or clinic based. Suggested changes to the MiNDToolkit intervention were made, including creating paper-based resources to supplement the online platform e.g., booklet with prompts of the strategies for HCP visits.

Conclusion

MND care is complex and requires a multidisciplinary team approach. The MiNDToolkit training and online platform demonstrate potential use as an intervention for increasing HCP awareness of behavioural symptoms of MND, and their management.



35. M50, CMAP50 and MUNIX200 are potentially new parameters to describe disease progression in Amyotrophic Lateral Sclerosis

Annekathrin Roediger (1*), Theresa Ebersbach (1), Robert Steinbach (1), Martin Appelfeller (1), Anke Tuemmler (1), Beatrice Stubendorff (1), Hubertus Axer (1), Otto W. Witte (1,2) and Julian Grosskreutz (3,4)

1 Department of Neurology, Jena University Hospital, Jena, Germany.

2 Center for Healthy Ageing, Jena University Hospital, Jena, Germany.

3 Precision Neurology, University of Lübeck, Lübeck, Germany.

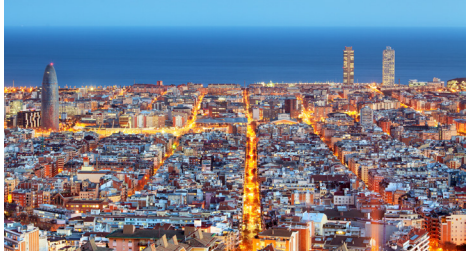
4 Cluster of Excellence Precision Medicine in Inflammation, University of Lübeck, Lübeck, Germany.

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal, neurodegenerative disease and shows a high variability due to different phenotypes and disease progression. Due to heterogenicity it is difficult to assess the efficacy of new drug therapies. Therefore, meaningful biomarkers are needed to evaluate disease progression in more detail. Motor unit number index (MUNIX) is a promising biomarker for disease progression and degeneration of lower motor neuron, but it is also influenced by ALS heterogenicity (e.g. onset of disease, phenotype, disease duration).

Method: MUNIX parameters of Musculi abductor pollicis brevis (APB), abductor digiti minimi (ADM) and tibialis anterior (TA) of 222 ALS patients were used to calculate M50, CMAP50 and MUSIX200 according to the D50 disease progression model. M50 and CMAP50 indicate the time in months from ALS symptom onset to lose 50% of MUNIX or CMAP in relation to the mean values of controls. MUSIX200 represents the time in months until doubling of the mean MUSIX of controls.

Results: In our ALS cohort, M50, CMAP50 and MUSIX200 preceded the loss of global motor function nearly one year (about 14 mo.). The M50 parameters differed significantly among disease aggressiveness subgroups ($p < 0.001$) regardless of disease accumulation. Also, ALS patients with a low M50 had a significantly shorter survival compared to patients with high M50 (32 versus 74 mo.).

Conclusion: We conclude that, M50, CMAP50 and MUSIX200 characterize the disease course in ALS in a new way and may be applied as early measures of disease progression.



36. Corticomuscular coherence in ALS during the performance of a motor task.

Saroj Bista* (1) Amina Coffey (1) Matthew Mitchell (1) Antonio Fasano (1) Stefan Dukic (1,2) Teresa Buxo (1) Marjorie Metzger (1) Prabhav Mehra (1) Eileen Giglia (1) Colm Peelo (1) Mark Heverin (1) Muthuraman Muthuraman (4) Lara McManus (1) Orla Hardiman (1,4) Bahman Nasseroleslami (1)

(1) Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.

(2) Department of Neurology, University Medical Centre Utrecht Brain Centre, Utrecht University, Utrecht, The Netherlands.

(3) Neural Engineering with Signal Analytics and Artificial Intelligence, Department of Neurology, University Hospital Würzburg, Germany.

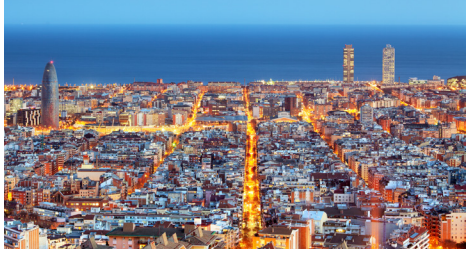
(4) Beaumont Hospital, Dublin, Ireland.

Background: Recent EEG studies in ALS have shown that there is altered functional connectivity across brain networks. It is not clearly understood how this abnormal connectivity influences the oscillatory drives from cortex to muscle, particularly in regions outside of the primary sensorimotor cortices. This can be assessed using corticomuscular coherence (CMC), which estimates the synchrony between signals recorded from the brain (EEG) and muscle (electromyography, EMG) in the frequency domain. The aim of this study was to use CMC to characterise the oscillatory drives between cortical regions and muscles during a motor task in ALS and to examine the relationship between CMC and the level of clinical impairment.

Methods: EEG (128 channels) and 8 bipolar EMG signals were recorded from 25 ALS and 22 age-matched controls during the performance of a simple precision grip visuomotor task. EEG signals were source reconstructed using the Fieldtrip toolbox in MATLAB. Corticomuscular coherence was estimated for the abductor pollicis brevis (APB), first dorsal interosseous (FDI), and flexor pollicis brevis (FPB) muscles and the following brain regions: primary motor (M1), primary sensory (S1), supplementary motor area (SMA), prefrontal (PFC), superior parietal (SPL) regions of both hemispheres.

Results: In the ALS group, smaller CMC peaks were observed in the alpha-band for FDI and contralateral M1, in the beta-band for FDI and bilateral SMA, and in the theta-band for FDI and ipsilateral SPL ($p<0.05$) when compared with the control group. Higher clinical impairment was also associated with higher peak CMC between APB and contralateral M1/S1 in the theta-band ($r=-0.62$, $p<0.01$ with M1 and $r=-0.56$, $p=0.01$ with S1, where clinical impairment was assessed using the ALSFRSR motor subscores).

Discussion: These results demonstrate that the oscillatory drives to the muscle, estimated using CMC, differ between ALS and control groups, and between different levels of clinical impairment. They suggest an atypical engagement of both contralateral and ipsilateral brain regions during motor activity in ALS, indicating the presence of pathogenic and/or adaptive/compensatory alterations in neural activity. The findings demonstrate the potential of CMC for identifying dysfunction within the sensorimotor networks in ALS.



37. Classification of ALS Patients Based on Resting-state EEG Trajectories: Clinical Relevance and Network Progression"

Marjorie Metzger (1, #)*, Stefan Dukic (1,2), Vladyslav Sirenko (1), Roisin McMackin (1,3), Eileen Giglia (1), Matthew Mitchell (1), Saroj Bista (1), Emmet Costello (1), Colm Peelo (1), Yasmine Tadjine (1), Serena Plaitano (1), Amina Coffey (1), Lara McManus (1), Prabhav Mehra (1), Darragh Walsh (1), Teresa Buxo, Antonio Fasano (1), Mark Heverin (1), Peter Bede (1), Muthuraman Muthuraman (4), Niall Pender (1,5), Orla Hardiman (1,5), Bahman Nasseroleslami (1)

1 Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Ireland.

2 Department of Neurology, University Medical Centre Utrecht Brain Centre, Utrecht University, Utrecht, The Netherlands.

3 Discipline of Physiology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland.

4 Neural Engineering with Signal Analytics and Artificial Intelligence, Department of Neurology, University of Wurzburg, Wurzburg, Germany.

5 Beaumont Hospital, Dublin, Ireland.

 # Joint First Authors

Background: As ALS is a heterogeneous condition, there is an urgent need for quantitative tools that can measure disease progression. Our previous studies using resting-state electroencephalography (RS-EEG) demonstrate stable network-based disease subphenotypes. Here we have classified longitudinal trajectories of spectral EEG measures to capture the progression of network disruption in ALS.

Aim: To define stable subgroups of ALS patients using data-driven clustering based on trajectories of resting-state spectral EEG measures over time and to determine whether such clusters can be linked to clinical presentation.

Methods: Resting-state EEG was used to estimate longitudinal cortical neural activity in 124 ALS patients. Features were extracted from the estimated linear trajectories (slopes and intercepts) of EEG spectral power and then selected using the Sparse Hierarchical Clustering algorithm. Patients were grouped into 2-7 subgroups based on their EEG data using hierarchical clustering. The statistical significance of the clusters was assessed, and the optimal number of clusters was determined accordingly. The stability of the clusters was then evaluated by iteratively excluding 10% of the participants [1] and by applying another clustering algorithm (Gaussian Mixture Model, GMM). Clinical profiles including motor, respiratory and cognitive decline as well as the site of onset and survival, were determined for the optimal subgroups.

Results: 6 relevant neurophysiological features containing information on widespread cortical high-gamma band (53-97 Hz) spectral power changes over time were further characterized. We identified 2 distinct progression trajectories of spectral power EEG (statistical analysis: p = 2·10^(-4)). The stability and propagation of clusters' labels to new participants, both reached 70% agreement with previously performed clustering. Significant differences (Mann-Whitney U test, FDR correction at 0.05) in clinical profiles were identified between the 2 groups of trajectories (survival: p = 0.004, ALSFRS-R subscores: p <0.05).

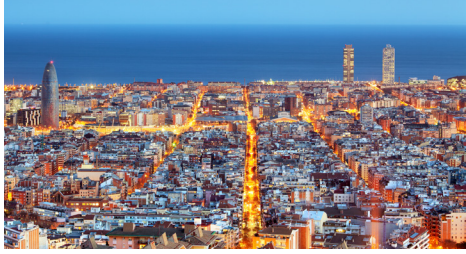
Discussion: This classification of longitudinal trajectories of a neurophysiological measure can provide clinically meaningful insights into disease trajectory.

Reference:

[1] S. Dukic et al., Brain, (2022), doi: 10.1093/brain/awab322.

Acknowledgement: We would like to thank patients and their families for their time, as well as Foundation Thierry Latran for the funding of this study.

Translational and Clinical Rapid Fire (2/2)



XII) isomiRs - a novel family of molecular biomarkers for ALS prognostication

Yahel Cohen* (1,2),Iddo Maggen (1,2), Nancy - Sarah Yacovzada(1,2), Andrea Malaspina (3), Pietro Fratta (3), Joen Wuu (4), Michael Benatar (4), Eran Hornstein (1,2).

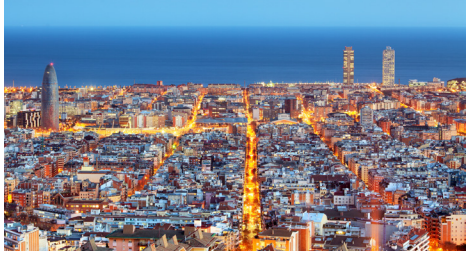
1 - Departments of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.

2 - Departments of Molecular Neurosciences , Weizmann Institute of Science, Rehovot, Israel.

3 - Department of Neuromuscular Diseases, UCL, Queen Square Institute of Neurology, London, UK.

4 - Department of Neurology, University of Miami, Miami, FL, USA

microRNAs are endogenous, non-protein coding, small RNAs. With Fratta and Malaspina labs we have reported the value of plasma microRNA-181 (miR-181) in ALS prognostication (Nature Neuroscience 2021). However, until recently, we were unable to analyze a much larger variety of microRNA isoforms, called isomiRs. Here, we develop a new bioinformatics technique for analysis of isomiRs. We reveal a novel panel of isomiRs, from a plasma cohort of 246 UK ALS patients, which are candidate ALS biomarkers with value in disease prognostication. Intriguingly, some of the isomiRs perform better as predictors than their canonical miRNA counterparts. Moreover, With Benatar, we were able to replicate our results for a specific isomiR of miR-339-5p in an independent American plasma cohort of 215 patients. This substantiate a first-of-its kind isomiR biomarker in ALS. This study features a conceptual and Innovative evaluation of a new family of molecular biomarkers in any disease. We hope that isomiRs will allow a more accurate ALS patient prognostication and promote clinical development.



XIII) Performance of serum neurofilament light chain in a wide spectrum of clinical courses of ALS – a cross-sectional multicenter study

Thomas Meyer (1,2), Erma Salkic (1), Torsten Grehl (3), Ute Weyen (4), Dagmar Kettemann (1), Patrick Weydt (5,6), René Günther (7,8), Paul Lingor (9), Jan Christoph Koch (10), Susanne Petri (11), Andreas Hermann (12,13), Johannes Prudlo (13,14), Julian Großkreutz (15), Petra Baum (16), Matthias Boentert (17), Moritz Metelmann (16), Jenny Norden (1), Isabell Cordts (9), Jochen H. Weishaupt (18), Johannes Dorst (19), Albert Ludolph (19,20), Yasemin Koc (1), Bertram Walter (1), Christoph Münch (1,2), Susanne Spittel (1,2), Marie Dreger (1)*, André Maier (1)*, Péter Körtvélyessy (1,21)*, *contributed equally

1) Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Neurology, Center for ALS and other Motor Neuron Disorders, Berlin, Germany.

2) APST Research GmbH, Berlin, Germany.

3) Alfried Krupp Krankenhaus, Department of Neurology, Center for ALS and other Motor Neuron Disorders, Essen, Germany.

4) Berufsgenossenschaftliches Universitätsklinikum Bergmannsheil, Department of Neurology, Center for ALS and other Motor Neuron Disorders, Bochum, Germany.

5) Bonn University, Department for Neurodegenerative Disorders and Gerontopsychiatry, Bonn, Germany.

6) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Bonn, Germany.

7) Technische Universität Dresden, Department of Neurology, Dresden, Germany.

8) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Dresden, Germany

9) Technical University of Munich, School of Medicine, Klinikum rechts der Isar, Department of Neurology, München, Germany.

10) Universitätsmedizin Göttingen, Department of Neurology, Göttingen, Germany.

11) Hannover Medical School, Department of Neurology, Hannover, Germany.

12) University of Rostock, University Medical Center, Department of Neurology, Translational Neurodegeneration Section "Albrecht-Kossel", Rostock, Germany.

13) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Rostock/Greifswald, Germany.

14) University of Rostock, University Medical Center Rostock, Department of Neurology, Germany.

15) Universitätsmedizin Schleswig-Holstein, Campus Lübeck, Department of Neurology, Lübeck, Germany.

16) Universitätsklinikum Leipzig, Department of Neurology, Leipzig Germany.

17) Universitätsklinikum Münster, Department of Neurology, Münster, Germany.

18) University Medicine Mannheim, Heidelberg University, Mannheim Center for Translational Medicine, Neurology Department, Division for Neurodegenerative Diseases, Mannheim, Germany.

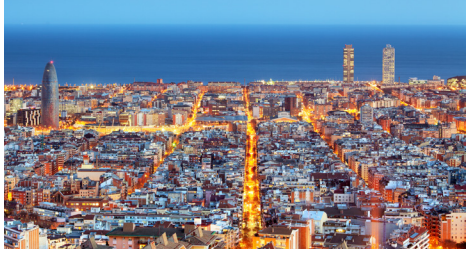
19) Ulm University, Department of Neurology, Ulm, Germany

20) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Ulm, Germany.

21) DZNE, Deutsches Zentrum für Neurodegenerative Erkrankungen, Research Site Magdeburg, Germany.

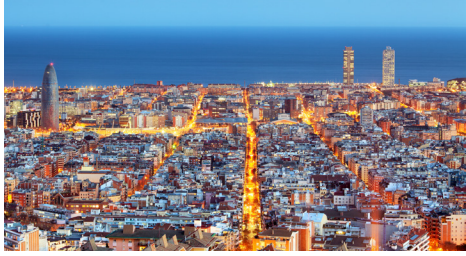
Objective: To assess the performance of serum neurofilament light chain (sNfL) in ALS in a wide range of disease courses – in terms of progression, duration, and tracheostomy invasive ventilation (TIV).

Methods: A prospective cross-sectional study at 12 ALS centers in Germany was performed. sNfL concentrations were age-adjusted using sNfL Z scores expressing the number of standard deviations from the mean of a control reference database and correlated to ALS duration and the ALS progression rate (ALS-PR), defined by the decline of ALS functional rating scale.



Results: In the total ALS cohort (n=1378) the sNfL Z score was elevated (3.04; 2.46-3.43; 99.88th Percentile). There was a strong correlation between the sNfL Z score with ALS-PR ($p<0.001$). In patients with long (5-10 years, n=167) or very long ALS duration (≥10 years, n=94) the sNfL Z score was significantly lower compared to typical ALS duration of <5 years (n=1059) ($p<0.001$). Furthermore, in patients with TIV, decreasing sNfL Z scores were found in correlation with TIV duration and ALS-PR ($p=0.002$; $p<0.001$).

Conclusions: The finding of moderate sNfL elevation in patients with long ALS duration underlined the favorable prognosis of low sNfL. The strong correlation of the sNfL Z score with ALS-PR strengthened its value as a progression marker in clinical management and research. The lowering of sNfL in correlation with long TIV duration could reflect either a reduction in disease activity or in the neuroaxonal substrate of biomarker formation during the protracted course of ALS.



XIV) Ultrasound-mediated blood–spinal cord barrier opening prolongs survival in an ALS mouse model

Anne-Sophie Montero (1,2,3), Ilyes Aliouat* (1,2,3,4), Michael Canney (5), Lauriane Goldwirt (6), Samia Mourah (6), Félix Berriat (4), Christian S Lobsiger (4), Pierre-François Pradat (7), François Salachas (4,7), Gaëlle Bruneteau (7), Séverine Boillée (4), Alexandre Carpentier (1,2,3)

(1) Sorbonne Université, Neurosurgery Department, AP-HP, Pitié-Salpêtrière Hospital; Paris, France.

(2) Advanced Surgical Research Technology Laboratory; Paris, France.

(3) Sorbonne Université, GRC 23, Brain Machine Interface, AP-HP, Pitié-Salpêtrière Hospital; Paris, France.

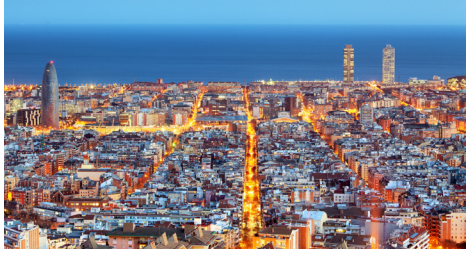
(4) Sorbonne Université, Institut du Cerveau – Paris Brain Institute – ICM - Inserm, CNRS, AP-HP, Hôpital de la Pitié-Salpêtrière, Paris, France.

(5) Carthera; Lyon, France

(6) AP-HP, Pharmacology Department, Hôpital de Saint-Louis; Paris, France.

(7) AP-HP, Centre de Reference Maladie Rare SLA, Neurology Department, Pitié-Salpêtrière Hospital; Paris, France.

The limited efficacy of therapies in clinical development for Amyotrophic Lateral Sclerosis (ALS) may be linked to lack of drug penetration to the affected motor neurons due to the blood-brain barrier (BBB) and blood-spinal cord barrier (BSCB). This work was intended to circumvent this limitation by using a system to transiently open the BSCB. The safety and efficacy of repeated short transient opening of the BSCB by low intensity pulsed ultrasound (LIPU, sonication) were studied in an ALS mouse model. The BSCB was disrupted using a 1 MHz ultrasound transducer coupled to the spinal cord, with and without injection of insulin-like growth factor 1 (IGF1), a neurotrophic factor that has previously shown efficacy in ALS models. Results in healthy mouse models demonstrate that the BSCB can be safely disrupted and IGF1 concentrations significantly increased after a single session of transient BSCB disruption (176 ±32 µg/g vs. 0.16 ±0.008 µg/g, $p<0.0001$). Five repeated weekly sonications performed in ALS mice demonstrated a survival advantage in mice treated with IGF1 and ultrasound (US) compared to treatment with IGF1 alone (176 vs. 166 days, $p=0.019$), but also in mice treated with ultrasound alone vs untreated mice (178.5 vs. 166.5 days, $p=0.018$). Thus, these results suggest a survival benefit of ultrasound alone, independently of IGF1 administration. Histological analysis revealed a modulation of glial cell reactivity and an increased CD4+ T-cell infiltration in the spinal cord of mice treated by US+IGF1, compared to treatment with IGF1 alone. As CD4+ T-cells are known to be protective in this ALS mouse model, the mechanism underlying the survival benefit may be an immunomodulatory effect of US or US+IGF1. These results show the first step towards a possible beneficial impact of transient BSCB opening implicating immune cells for ALS therapy, as well as efficient BSCB opening for drug delivery.



XV) Motor system connectivity in ALS: A corticomuscular magnetoencephalography study

Katie Yoganathan (1*), Michael Trubshaw (1), Irene Echeverria-Altuna (2,3), Oliver Kohl (2), Thanuja Dharmadasa (1), Nahid Zokaei (2), Andreas Themistocleous (1), Charlotte Stagg (2), Mark Woolrich (2), Anna C Nobre (2,3), Kevin Talbot (1), Alexander G. Thompson (1) and Martin R. Turner (1).

1. Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, Oxfordshire, UK.

2. Oxford Centre for Human Brain Activity, Wellcome Centre for Integrative Neuroimaging, University of Oxford, Oxford, Oxfordshire, UK.

3. Department of Experimental Psychology, University of Oxford, Oxford, Oxfordshire, UK.

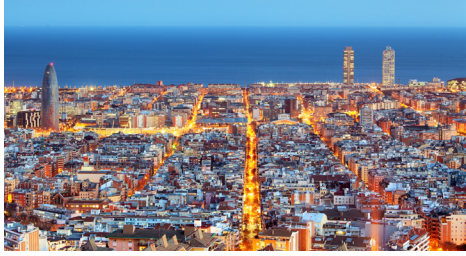
Introduction: Biomarkers of disease activity in ALS are needed to allow objective and sensitive detection of therapeutic benefit and accelerate the process of drug discovery. ALS is motor system disorder spanning cortex to muscle. Magnetoencephalography (MEG) is the most sensitive, non-invasive assessment measure of regional cortical neurophysiology. Corticomuscular coherence (CMC) reflects the functional coupling of cortical oscillations and downstream muscle activity in the search for a more holistic biomarker of motor system dysfunction in ALS.

Hypothesis: MEG-led measures of motor system functional connectivity can identify specific disrupted neural dynamics associated with ALS pathology.

Methods: In an ongoing study, data were available from 15 ALS patients and 15 healthy age-similar controls (HC). Participants underwent clinical evaluation with clinical assessments and a standardised MEG protocol involving a novel gripper task. Muscle contraction was measured using bipolar surface EMG recordings at both forearms. All participants performed 240 trials of the gripper task bilaterally, and 120 trials unilaterally on each side.

Results: During gripper-based muscular contraction, beta-band frequency CMC in the motor cortex was significantly reduced in ALS patients compared to healthy controls (cluster-based permutations between 8 – 25 Hz). There were no significant differences between absolute grip strength of the ALS patients and HC. Beta-band power differences were seen in the peri-response desynchronisation, and post-movement rebound. A reduction of CMC between cortex and contralateral muscle was also evident when considering its topographical distribution using localisation analysis. In both groups, coherence was localised to the same contralateral motor channels, but was considerably weaker in ALS disease compared to HC participants.

Conclusion: Beta-band CMC is a relatively easily acquired biomarker of early ALS motor system dysfunction. Albeit at the group level currently, there are immediate opportunities for its exploration as a secondary outcome measure in therapeutic trials, but in future work also as a potential pre-symptomatic biomarker in carriers of pathological variants linked to ALS.



XVI) Dysfunction Of Cortical Inhibitory Interneurons In Amyotrophic Lateral Sclerosis

Cristina Benetton* (1), Pierre-François Pradat (1, 2), Véronique Marchand-Pauvert (1), Alexandra Lackmy-Vallee (1)

(1) Sorbonne Université, Inserm, CNRS, Laboratoire d'Imagerie Biomédicale, LIB, Paris, France.
(2) Neurologie, AP-HP, Hôpital Pitié-Salpêtriére, Paris, France.

Background: Sporadic (s) and familial (f) forms of Amyotrophic Lateral Sclerosis (ALS) are characterized by perturbed excitation/inhibition (E/I) input balance to motoneurons. Previous studies using paired-pulse TMS (ppTMS) had shown that ALS is distinguished by a depressed Short-Intracortical Inhibition (sICI) and an enhanced Intracortical Facilitation (ICF). To shed light on mechanisms causing neuron excitability disorders, it is crucial to consider afferent interneurons that project onto UMN.1 2

Objective: Our aim is to investigate the degree of excitability of inhibitory interneurons projecting to UMN in early diagnosed sALS patients as compared to healthy controls (HS). Our research hypothesis is to find a perturbed sICI in ALS patients as compared to HS across different conditions (rest vs. tonic contraction).

Methods: Data were collected on 12 sALS patients and 16 HS. ppTMS was delivered over the primary motor cortex to evoke sICI in abductor digit minimi (ADM). To stress changes in inhibitory interneuron excitability, sICI was evaluated in different conditions: 1) Test TMS intensity was set at 1.2 of the resting motor threshold (RMT). Two intensities of conditioning TMS were tested (0.7 X RMT and 0.7 of the active MT, AMT) 2) sICI was compared at rest and during 10% of the maximal voluntary contraction.3 5

Results and Conclusions: The RMT was higher in sALS patients compared to HS. Given that test TMS intensity was stronger in sALS patients, the amplitude of the test motor evoked potential (MEP) was about 10% of the maximal motor action potential (Mmax) while it was about 5% Mmax in HS. Thus, the test MEP in ALS patients was optimal to follow the modulations of sICI across conditions. At rest sICI was weaker in ALS patients than in HS. Interestingly, we found that sICI was not modulated by the tonic contraction in ALS patients while in HS the contraction depressed the sICI. These finding suggest a dysfunction of inhibitory interneurons afferent to UMN, at early stage of ALS. This opens avenues to new approaches to counteract the imbalance of E/I observed in the motor cortex in attempt to slow down UMN loss in ALS.3 4

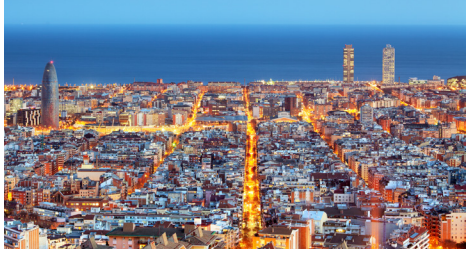
1 Vucic S, Ziemann U, Eisen A, et al. J Neurol Neurosurg Psychiatry 2013;84:1161-1170

2 Sangari S, et al. Clin Neurophysiol 2016;127,4:1968-1977

3 Lackmy-Vallee A, et al. Eur J Neurosci 2012;35:457–467

4 Lackmy A, Marchand-Pauvert V, Clin Neurophysiol 2010;121:612-621

5 Kujirai T, et al. Journal of Physiology 1993 ;471 :501-5



XVII) NERVE EXCITABILITY DISENTANGLED: HYPEREXCITABILITY IN ALS IS DRIVEN BY ALTERED SLOW POTASSIUM CHANNEL KINETICS

Diederik Stikvoort Garcia(1), Boudewijn Sleutjes(1), Stephan Goedee(1), Leonard van den Berg(1)

(1)Department of neurology, University medical center Utrecht, Utrecht, The Netherlands

Background:

Changes in excitability of motor axons in MND are suggested to represent an early step in the neurodegenerative cascade. Ion-channel dysfunction is believed to play a crucial role in the observed excitability patterns. In this study, we utilize a novel approach to better disentangle the biophysical origins of motor nerve excitability changes in patients with ALS, and examine their association with several clinical characteristics.

Methods:

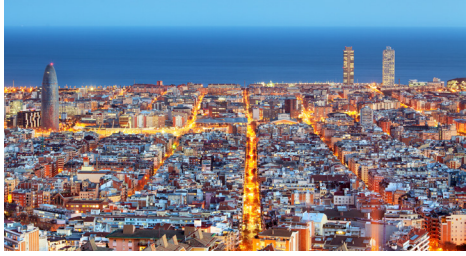
We prospectively recruited 167 MND patients during their first diagnostic workup and 37 age-gender matched healthy controls. Participants underwent standardized nerve excitability tests on the median nerve and thenar muscles. Clinical reference measures included: ALSFRS-R scores, survival, C9orf72 mutation status, onset region, presence of fasciculations and motor unit number estimates (MUNE) of the examined muscle. Nerve excitability recordings were age-gender corrected and z-transformed with respect to the healthy controls, after which principal components (PCs) were derived. These PCs leverage the correlations between measurement points. Associations between PCs and clinical measures were established. Then, we simulated changes in PCs to obtain novel excitability curves on which we fitted a well-established nerve model to generate insight into the origin of the excitability changes.

Results:

We retained 4 PCs explaining 64% of the variance in the excitability measures (26%, 18%, 11%, 9%, resp.). Modelling indicated that PC1 was mainly associated with voltage-gated independent properties, including resting membrane potential. Under prevailing MUNE reduction, changes in PC1 indicated decreased excitability. PC2 had the strongest relation with slow potassium gating kinetics. In contrast, reduced MUNE was associated with decreased PC2, indicative of increasing excitability. Increased PC2 was also associated with shorter survival (HR [95%CI]=1.06 [1.02-1.10], $p<0.01$) and faster decrease in ALSFRS-R at follow-up ($p<0.01$), while C9orf72 patients had lower PC2 values ($p<0.05$). PC3 was indicative of axonal refractoriness. Despite absence of clear neural origin, this value was increased patients with C9orf72 mutations ($p<0.05$), bulbar onset ($p<0.05$) and fasciculations ($p<0.01$). PC4 was best explained by sodium channel properties and ion-concentrations, but was not associated with any clinical measure.

Discussion:

We provide in vivo evidence that increased and decreased excitability are processes that can o



XVIII) Social Cognition Impairment in Amyotrophic Lateral Sclerosis

Sara Kadenšek* (1), Vita Štukovnik (2), Janez Zidar (1), Blaž Koritnik (1)

(1) Ljubljana ALS Centre, Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia.
(2) Department of Psychology, Faculty of Arts, University of Maribor, Slovenia.

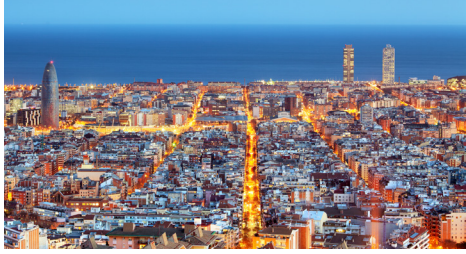
Introduction: Cognitive changes in patients with amyotrophic lateral sclerosis (ALS) may present as deficits in social cognition (SC). The aim of this research was to examine social cognitive abilities in healthy controls and patients with ALS by utilizing three distinct evaluations, and to determine whether there were any variations in results. This study also investigated differences in SC between ALS patients with bulbar (ALS-B) and spinal onset (ALS-S), and association of the degree of severity of motor impairment to SC impairment.

Methods: Patients with ALS (n=30) and age, sex and education matched controls (n=29) underwent a SC assessment through the Social Cognition scale on Edinburgh Cognitive and Behavioral ALS Screen (SC-ECAS), the Reading the Mind in the Eyes Test (RMET), and the Edinburgh Social Cognition Test (ESCoT).

Results: ALS patients showed significantly worse performance compared to controls in SC-ECAS (p=.024) and ESCoT (p=<.001). Despite patients reaching lower average score on RMET than controls, there were no significant differences between groups (p=.226).

We found no correlation between severity of ALS based on Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) and social cognition impairment. ALS-B showed significantly worse performance compared to patients with ALS-S on RMET (t(7.74)=-2.92, p=.021), and on ESCoT (t(9.36)=-2.79, p=.020). Despite ALS-B reaching lower average score on SC-ECAS than ALS-S, there were no significant differences between groups (p=.270).

Conclusions: Our study indicates that ALS patients show impairment in SC performance. However, there were no significant differences between controls and ALS patients on RMET. Our findings did not reveal any indication that greater levels of motor impairment in ALS cases are linked to more severe SC deficits. We have confirmed a greater SC impairment in ALS-B compared to ALS-S; yet, we found no significant differences on SC-ECAS.



XIX) Motor band sign is a specific marker of ALS and corresponds topographically to motor symptoms

Charlotte Zejlon(1,2)*, Stefan Sennfält(2,3), Johannes Finnsson(1,2), Bryan Connolly(1,2), Sven Petersson(1,4), Tobias Granberg(1,2), Caroline Ingre(2,3)

1. Department of Neuroradiology, Karolinska University Hospital, Stockholm, Sweden.
2. Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden.
3. Department of Neurology, Karolinska University Hospital, Stockholm, Sweden.
4. Department of Medical Radiation Physics and Nuclear Medicine, Stockholm, Sweden.

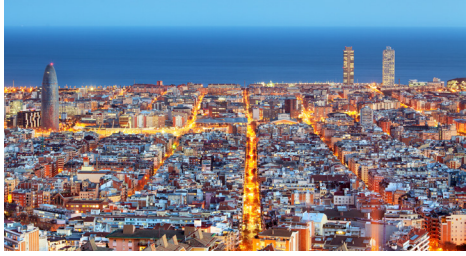
Background: A hallmark feature of ALS is the simultaneous involvement of upper motor neurons and lower motor neurons; evidence of both is a prerequisite for the diagnosis. There are few methods of detecting upper motor neuron dysfunction, which contributes to diagnostic delays. Meanwhile, brain MRI can demonstrate neurodegenerative iron accumulation in the motor cortex, known as the motor band sign (MBS).

Aim: To evaluate the sensitivity and specificity of the MBS for ALS and its correlation to focal motor weakness using a novel visual rating scale.

Methods: This prospective study consecutively included 117 ALS patients (66.3±12.3 years, 66 males), 79 ALS mimics (62.5±15.8 years, 46 males) and 31 age- and sex-matched neurologically healthy controls (63±14.3 years, 10 males). A 3 Tesla Siemens PrismaFit scanner with a 64-channel head coil was used to perform 3D susceptibility-weighted imaging. Three raters with varying experience (resident in radiology, fellow in neuroradiology and neuroradiologist) assessed susceptibility in the motor cortex based on a novel visual rating scale. Total and regional (medial, lateral and hand knob) MBS scores were calculated and compared between groups. Associations to the revised ALS functional rating scale (ALSFRS-R) and its subscores for fine motor, gross motor, bulbar, and respiratory function were assessed using regression analysis.

Results: Positive MBS was seen in 69 ALS patients (59%) compared to in 1 control (3.2%) and 7 ALS mimics (8.9%). This translates to a sensitivity of 59% and a specificity of 90% vs. mimics and 97% vs. controls. Higher total MBS scores were significantly associated with lower total ALSFRS-R scores (std. δ =-0.30, P=0.018) but not with progression rate (P=0.64). Topographically, there was a strong association between medial MBS and gross motor dysfunction (std. δ =-0.58, P=0.002), between hand knob MBS and fine motor dysfunction (std. δ -0.69, P=0.007) and between lateral MBS and bulbar symptoms (std. δ =-0.62, P<0.001).

Conclusions: Primary motor cortex susceptibility has high specificity but relatively low sensitivity for identifying ALS. Regional MBS scores are associated with focal motor weakness, corresponding topographically to the somatotopic organization of the primary motor cortex. These findings suggest that the MBS may be a diagnostic imaging biomarker for ALS. Its predictive value and potential role for clinical trials and treatment monitoring remains to be studied.



XX) Investigating cognitive endophenotypes and presymptomatic cognition in unaffected relatives of familial ALS patients: a longitudinal study

Colm G Peelo* (1,2), Sarah Darcy (1,3), Emmet Costello (1,2), Marie Ryan (1,3), Mark Heverin (1), Orla Hardiman (1,3), & Niall Pender (1,2)

1 Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Dublin, Ireland.

2 Department of Psychology, Beaumont Hospital Dublin, Dublin, Ireland.

3 Department of Neurology, Beaumont Hospital Dublin, Dublin, Ireland.

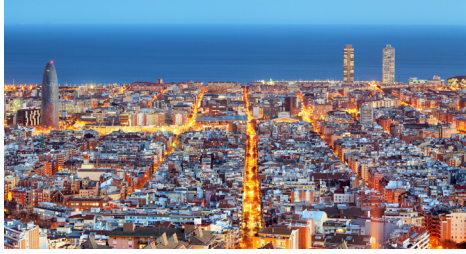
Background: C9orf72 is the most common genetic cause of familial-ALS (fALS). It is associated with cognitive and behavioural symptoms in ALS patients. More recently, candidate cognitive endophenotypes have been identified in unaffected fALS relatives[1], independent of C9orf72-gene status. This longitudinal study aims to assess 1) the stability of the candidate cognitive endophenotype & 2) examine longitudinal cognitive performance in unaffected fALS relatives, including C9orf72-gene carriers.

Methods: A preliminary sample of 22 unaffected first- and second-degree relatives of fALS patients (10 C9orf72-positive) and 26 healthy controls completed the ECAS and FAS phonemic verbal fluency task at two time points, 4 years apart.

Results: Groups were matched for sex ($p=0.56$) and education ($p=0.4$) but not age ($p<0.001$) or premorbid IQ ($p<0.001$). Relatives scored significantly lower than controls on ECAS Total ($b=-6.37$, $p=0.01$) and ECAS ALS-Specific domain ($b=-4.242$, $p=0.04$). This effect remained when controlling for premorbid IQ, age and education. Although relatives trended towards having lower scores on the FAS verbal fluency task, there was no main effect of group in this smaller sample ($b=-0.314$, $p=0.39$). There was a significant main effect of time on ECAS Total score ($p=0.02$) and FAS z-score ($p=0.01$), with both groups improving. However, there was no significant interaction effects of group and time for any variable ($p>0.05$). C9orf72-gene carriers ($n=10$) did not differ from non-carriers ($n=11$) on any domain ($p>0.05$).

Conclusion: Unaffected fALS relatives perform worse than controls on the ECAS, especially in ALS-Specific domains, and this is consistent over time. C9orf72-status had no bearing on cognitive performance in unaffected fALS relatives. These preliminary findings support previous work pointing to a cognitive endophenotype in kindreds of fALS patients, which may suggest an increased disease liability in this cohort.

1Costello et al.,2018



XXI) Cortical network dysfunction in ALS using task-free magnetoencephalography

Michael Trubshaw(1,2)*, Chetan Gohil(1), Katie Yoganathan(1,2), Evan Edmond(1,2), Malcolm Proudfoot(1,2), Mark Woolrich(1), Martin R. Turner(2)

1. Wellcome Centre for Integrative Neuroimaging, University of Oxford, Oxford, United Kingdom
2. Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

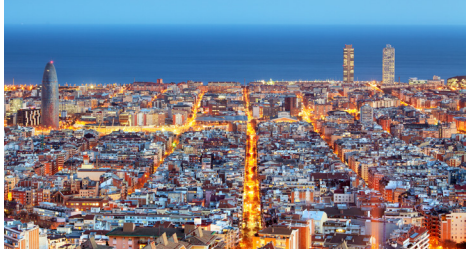
ALS involves complex primary pathological and compensatory dysfunction of cerebral networks. Callosal (inter-hemispheric pathway) pathology has been a consistent observation in histological and MRI studies in ALS. Magnetoencephalography (MEG) is a brain imaging technique which non-invasively measures the micro-magnetic fields generated by oscillatory brain activity. Modern computational techniques allow analysis of this data to provide highly temporally and spatially localised information about cortical neurophysiology. Task-free, resting-state MEG offers unique insight into cerebral pathophysiology by revealing dynamic, network-level changes in oscillatory brain power and connectivity.

Thirty-six non-demented patients with apparently sporadic ALS and 51 age- and gender-matched controls underwent an 8-minute resting-state MEG recording and structural MRI scan. We calculated oscillatory cortical power, connectivity and complexity measures in 52 regions and 5 canonical frequency bands (δ , δ , δ , δ , δ). We used maximum statistic permutations tests to look for differences between groups, and the effect of disability (ALSFRS) on brain function, correcting for age, gender and handedness.

ALS patients showed decreased beta power in sensorimotor regions (p=0.034) and increased gamma power in frontotemporal regions (p=0.015). Frontotemporal activity complexity was also increased in ALS patients (p=0.008). Higher levels of ALS patient disability were associated with increased power in these same regions (p=0.007) and increased frontotemporal connectivity overall (p=0.005) but relatively more with the contralateral hemisphere (p=0.034).

A fall and regional shift of sensorimotor beta power is the consistent underpinning of ALS cerebral pathophysiology. Increased power, connectivity and complexity of brain activity in frontotemporal regions may reflect a distinct process within ALS and FTD's pathological spectrum, or compensatory response to primary motor system disintegration. Altered functional laterality may reflect specific damage to inter-hemispheric fibres of the corpus callosum. MEG may offer a biomarker for disease progression in ALS and secondary outcome measure in therapeutic trials. The extent to which MEG network changes occur pre-symptomatically requires dedicated exploration in carriers of pathological genetic variants.

Session 9. Pre-symptomatic phenotype and cognition



38. Phenoconversion of asymptomatic genetic mutation carriers to ALS/FTD: a longitudinal neuroimaging study

Kevin van Veenhuijzen*(1), Henk-Jan Westeneng (1), Abram Nitert (1), Harold Tan (1), Annebelle Michielsen (1), Jan Veldink (1), Leonard van den Berg (1)

Department of Neurology, Brain Center, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

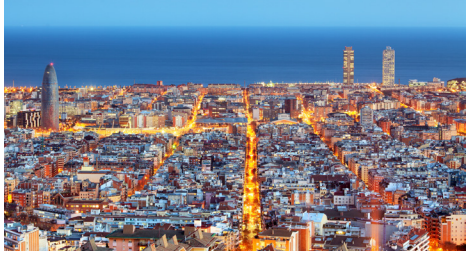
Introduction: Neuroimaging studies have demonstrated structural alterations both in ALS patients and asymptomatic carriers of C9orf72 repeat expansion. Usually subjects enroll in studies after diagnosis, with no scans available from their presymptomatic phase. Consequently, little is known about changes in the brain around time of phenoconversion from asymptomatic to ALS or FTD.

Objective: To investigate the onset, progression and extent of neurodegeneration before and after phenoconversion of mutation carriers to ALS/FTD using longitudinal MRI.

Methods: We included six subjects who developed ALS/FTD during the study ("converters"). We also included 47 asymptomatic family members (AFM) with C9orf72 repeat expansion and 359 population-based controls. All participants were scanned using 3T MRI, extracting vertex-wise cortical thickness. A mixed effects model was fitted at each vertex using scans of AFM (n=102), incorporating kinship as random effect, to predict cortical thickness. This model was applied to converter scans to calculate residuals (as measure of atrophy). Threshold-free cluster enhancement (TFCE) enhanced clusters of significantly deviant residuals. The mass of each cluster was calculated by summation of the enhanced residuals of all vertices within it. The same steps were taken with control scans (n=359) to create a null distribution of cluster masses. Clusters in scans of converters with a mass <2.5th percentile of the null distribution were deemed significantly atrophied. Thickness and area of these clusters were followed longitudinally.

Results: Mean time between first scan and symptoms was 36 months (range 2-68). In all cases, atrophy was present before symptom onset. All converters had a similar pattern of cortical atrophy on their 22 scans. The precentral gyrus (including the motor cortex), superior and inferior frontal and orbitofrontal cortex, precuneus and cingulate gyrus were most prominently involved. Total surface area of significantly atrophied clusters increased over time, and mean cortical thickness decreased. This was mostly driven by the precentral and cingulate gyrus, and superior frontal cortex. The rate of these alterations did not change after phenoconversion.

Conclusion: Phenoconverters show a consistent pattern of atrophy that is distinguishable from the impairments already found in asymptomatic carriers of C9orf72 repeat expansion. This pattern expands gradually and can precede symptom onset by months to years.



39. Investigation of cognitive networks in asymptomatic C9orf72 repeat expansion carriers using high-density EEG

Stefan Dukic* (1)(2), Robin Jansen (1), Roisin McMackin (2), Boudewijn T.H.M. Sleutjes (1), Bahman Nasseroleslami (2), Orla Hardiman (2)(3), Leonard H. van den Berg (1)

1 Department of Neurology, University Medical Centre Utrecht Brain Centre, Utrecht University, Utrecht, The Netherlands.

2 Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, University of Dublin, Ireland.

3 Department of Neurology, Beaumont Hospital, Dublin, Ireland.

Background

While previous studies of asymptomatic carriers of the C9orf72 hexanucleotide repeat expansion have reported alterations in both motor and cognitive domains across multiple modalities[1], early electrophysiological changes are still largely unexplored. Timely detection and characterisation of disease manifestations can lead to new therapeutic strategies that are based on targeted treatments. Here, we aim to evaluate changes in the cortical networks in C9orf72 asymptomatic family members (AFM) of familial ALS patients using high-density electroencephalography (EEG).

Methods

High-density EEG was recorded during the frequency mismatch negativity (MMN) paradigm in 15 C9+ AFM and 21 C9- AFM. The MMN paradigm entrains frontotemporal networks during involuntary attention shift [2]. In this paradigm, participants are asked to watch a silent movie, while a random sequence of two tones (1320 standards and 150 deviants) are being administered through headphones.

Using the average difference wave (deviant-standard) from the frontal midline electrodes, we evaluated the event-related potentials associated with the paradigm: early MMN and P3a; the former known to be diminished in ALS [3]. Statistical comparisons were performed using linear mixed models, while incorporating pedigree information to correct for familial relations and lessen the effects of other genetic factors.

Results

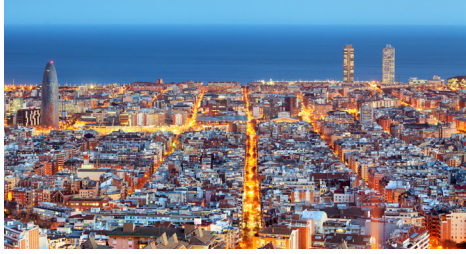
In both groups, as expected, the difference wave revealed the early MMN (110-175 ms post-stimulus) and the P3a (260-340 ms) peak. The early MMN was significantly increased ($P = 0.006$), while the P3a activation was significantly decreased ($P = 0.009$) in C9+ AFM.

Discussion

These results show the potential of EEG to capture functional brain changes associated with the C9orf72 repeat expansion and could suggest impaired excitatory/inhibitory balance in the brain [4]. Identification and characterisation of biomarkers linked to the early development of ALS can aid in early diagnosis, treatment strategy development, and enhance our understanding of the underlying (patho)physiological processes

References

1. Rangariroyashe Ch, et al. Journal of Neurology. 2020
2. Fitzgerald K, et al. Frontiers in Psychiatry. 2020
3. Iyer P, et al. Frontiers in Neurology. 2017
4. Wacongne C, et al. Journal of Neuroscience. 2012



40. Cognitive impairment and capacity to consent to clinical trials in ALS

Debbie Gray* (1, 3, 4), Luke Williams (2), Judith Newton (3, 4, 5), Suvankar Pal (3, 4, 5), Siddharthan Chandran (3, 4, 5), CARE-MND Consortium (3, 4, 5), Sarah MacPherson (1), Sharon Abrahams (1, 3, 4).

1 Department of Psychology, University of Edinburgh, Edinburgh, UK.
2. NHS Ayrshire and Arran, Crosshouse Hospital, Kilmarnock, UK
3. Euan MacDonald Centre for Motor Neurone Disease Research, Royal Infirmary of Edinburgh, Edinburgh, UK.
4. Anne Rowling Regenerative Neurology Clinic, Royal Infirmary of Edinburgh, Edinburgh, UK.
5. Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK.

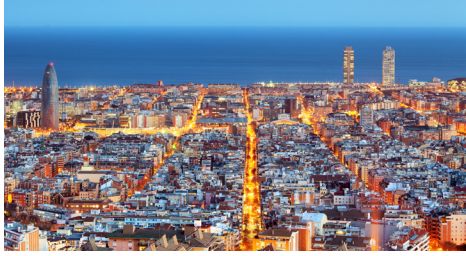
Introduction: pwALS must make important decisions throughout their disease, such as whether to participate in a clinical trial. However, up to 50% show experience cognitive and behavioural changes that could affect their decision-making ability. A mental capacity assessment evaluates a person's ability to understand, retain, weigh up the pros and cons, and communicate information relevant to a specific decision. Impairments in executive functioning, language, social cognition, and motivation could manifest as difficulties in attention, planning, weighing up options, considering consequences, understanding others' thoughts and engagement in the decision-making process.

Objectives: To explore if capacity to consent to clinical trials is affected in some pwALS and identify factors associated with decision-making.

Methods: 24 pwALS and 29 non-ALS individuals (with their study partners) participated in 2 semi-structured interviews over 2 weeks, either in-person or online. Mental capacity interviews were based on MacArthur Competency Assessment Tool – Clinical Research (MacCAT-CR) and Treatment (MacCAT-T). These were adapted for pwALS and included 2 hypothetical scenarios; a clinical trial and a holiday decision. Other factors assessed included cognition and behaviour (Edinburgh Cognitive and Behavioural ALS Screen; ECAS), apathy (Brief Dimensional Apathy Scale; b-DAS), mood (modified Hospital Anxiety and Depression Scale; HADS) and functional ability (ALS Functional Rating Scale-Revised; ALSFRS-R).

Results: In pwALS, 1/24 had cognitive impairment only (CI), 8/24 had behaviour impairment only (BI) and 2/24 had both (CBi). In the clinical trial scenario, 15/24 (4/8 with BI, and 2/2 with CBi) struggled to understand the purpose of the drug trial. By failing this interview component they would be judged as not having capacity to make this decision. However, all pwALS had the capacity to decide about the holiday. Further data will be presented, including quantitative data for between group comparisons and correlational analyses.

Conclusions: The findings have implications for clinical trial recruitment. These preliminary results show that some pwALS may have problems in understanding the information given and as such may not have capacity to make that decision. The findings will also help to identify whether this is related to CI, BI and mood. It will also identify individuals who may require support to make informed choices to ensure they maintain autonomy.



41. Premorbid brain structural variation influences risk of ALS

Alexander G Thompson* (1), Bernd Taschler (1), Stephen Smith (1), Martin R Turner (1)

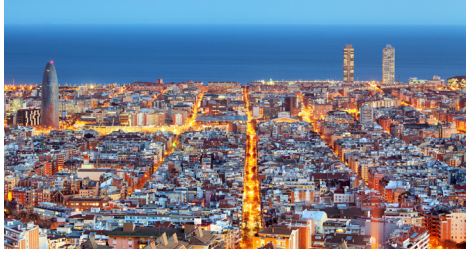
Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK.

Amyotrophic lateral sclerosis (ALS) is a disease of the motor network associated with alterations in brain structure and functional connectivity. Whether such changes have a causal role in ALS is not known. This study considered the causal effects of structural and functional MRI brain scan-derived phenotypes (IDPs) on the risk of ALS using a two sample Mendelian Randomization approach, as well as shared genetic risk between IDPs and ALS.

Genetic instruments from genome-wide association studies (GWAS) of 2881 IDPs were selected using summary-level data from healthy individuals aged 46-82 enrolled prospectively in UK Biobank, sampled and scanned at baseline (n=33,224). Genetic association data for ALS were obtained from independent GWAS (n=20,806). Causal associations were estimated using inverse variance weighted analysis of strong (F-statistic > 10) and independent (linkage dysequilibrium r2<0.001) genetic instruments. IDPs (and contralateral hemispheric IDP, where relevant) with a raw p<0.01 and concordant direction of association following validation in a second ALS GWAS were selected for further analysis. Sensitivity analyses were performed using methods considering horizontal pleiotropy, potential confounding associations of LDL cholesterol levels, smoking, physical exercise and body mass index, instrument heterogeneity and reverse causality. Linkage dysequilibrium score regression was used to estimate the genetic correlation of ALS with the IDP set.

Increased white matter volume in the cerebral hemispheres was causally associated with ALS (left hemisphere δ=0.26, 95% CI 0.13 to 0.38 p<0.001; right hemisphere δ=0.24, 95% CI 0.11 to 0.36, p<0.001). Weaker causal associations were observed for brain stem grey matter volume (δ=0.20, 95% CI 0.05 to 0.35, p=0.009), the parieto-occipital white matter surface (left hemisphere δ=0.24, 95% CI 0.09 to 0.40, p<0.001; right hemisphere δ=0.31, 95% CI 0.15 to 0.48, p<0.001) and the left ventral anterior nucleus of the thalamus (δ=0.27, 95% CI 0.10 to 0.44, p=0.002). Findings were independent of age and sex. Genetic correlation was observed between ALS and the diffusion imaging-derived intracellular volume fraction (0.311, p=0.001) and isotropic free water volume fraction (0.265, p=0.003) within the posterior limb of the right internal capsule.

This study provides evidence that premorbid brain structure, in particular white matter volume, reflects biological processes that contribute to the risk of sporadic ALS.



42. Brain "neurovascular coupling" in amyotrophic lateral sclerosis: the link with cognitive impairment

Minoo Sharbafshaaer 1*, Antonietta Canna 1, 2, Carla Passaniti 1, Fabrizio Canale 1, 3, Giulia D'Alvano 1, 3, Francesca D'Ammora 1, 3, Mattia Siciliano 1, 4, Gioacchino Tedeschi 1, 3, Fabrizio Esposito 1, Francesca Trojsi 1, 3.

1- Department of Advanced Medical and Surgical Sciences, MRI Research Center, Università degli Studi della Campania Luigi Vanvitelli, 80138 Naples, Italy.

2- Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, USA.

3- First Division of Neurology, Università degli Studi della Campania "Luigi Vanvitelli", 80138 Naples, Italy.

4- Neurosciences Research Centre, Molecular and Clinical Sciences Research Institute, St George's University of London, London, United Kingdom.

Introduction: Amyotrophic Lateral Sclerosis patients may show decreased spontaneous brain activity on resting-state functional MRI (RS-fMRI), as indexed by the ALFF, ReHo, and DC measures. Among the most studied resting-state networks (RSNs) in ALS, functional connectivity was frequently revealed to be impaired in Default Mode Network (DMN). Novel insights regarding functional/metabolic alterations of the brain in ALS may be related to the investigation of "neurovascular coupling" (NVC). We aim to explore the potential NVC alterations across different RS-fMRI networks (constituent node regions) via the above spatial combination of "cerebral blood flow" (CBF) and ALFF maps, as could be respectively derived from ASL, BOLD RS-fMRI measurements, in samples of ALS patients compared to healthy controls.

Method: Fifty-one right-handed ALS patients and twentyδ five rightδ handed HCs were enrolled at the First Division of Neurology of the University of Campania "Luigi Vanvitelli" (Naples, Italy). ALS patients were screened by clinical (ALSFRS-R, King's staging, UMN burden) and neuropsychological (Italian version of the Edinburgh Cognitive and Behavioural ALS Screen, ECAS) scales. MRI images were acquired on a 3 Tesla scanner equipped with a 32-channel parallel head coil.

Result: No patient was affected by dementia, and 17 patients had cognitive impairment (ALSci) according to Strong criteria (2017). Among 7 RSNs, a statistically significant reduction in NVC was found in the DMN ($F=8.16$, $p=0.005$), when NVC was computed as a correlation between ALFF values and CBF in ALS patients compared to the HC. As for the correlation between NVC (ALFF-CBF) in DMN and the collected ECAS scores, we found significant correlations between NVC in DMN and executive function subscore ($r=0.40$, $p=0.01$), memory subscore ($r=0.32$, $p=0.04$), visuospatial ability subscore ($r=0.40$, $p=0.01$), and ALS-non-specific subscale ($r=0.4$, $p=0.01$).

Conclusions: Reduction of brain NVC in DMN may reflect abnormalities of the neurovascular unit together with alterations of functional connectivity in extra-motor areas in non-demented ALS patients. Our findings suggest that significant changes in NVC occur outside motor areas in correlation with changes in cognitive performance across several cognitive domains in ALS patients. NVC measures might represent a valuable tool for exploring the early signature of cognitive/extra-motor impairment in ALS, thus allowing us to better characterize the complex phenotypes.

ENCALS

European Network to Cure ALS

ENCALS meeting 2023

Barcelona, Spain • 11th–14th July