Meeting 2017

# ENCALS

European Network to Cure ALS
18 - 20 May, Ljubljana, Slovenia

# Book of abstracts

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**European Network to Cure ALS**

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**Ljubljana ALS Centre, Institute of Clinical Neurophysiology**
**University Medical Centre Ljubljana, Slovenia**
**Slovenian Society of Clinical Neurophysiology**

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# Welcome Address

Dear colleagues,

Amyotrophic lateral sclerosis (ALS) is a devastating disease that is still lacking an effective cure. In recent year, research advances offer hope that ALS will eventually become a preventable and treatable disease. Patients, researchers and clinicians worldwide have joined forces in various international projects to make progress towards this goal. European Network to Cure ALS (ENCALS) is a network of ALS centres in Europe that supports such collaborations. 2017 is an exciting year for the ALS community since positive clinical drug trials are promising new treatments after more than two decades.

The Ljubljana ALS Centre at the Institute of Clinical Neurophysiology, University Medical Centre Ljubljana was established in 2002 to offer multidisciplinary clinical care for the Slovenian patients with ALS. In the past 15 years, we have cared for more than 500 patients. Our research encompasses neurophysiology, neuroimaging, genetics and epidemiology of ALS. We are collaborating with other Slovenian and international research centres.

It is my pleasure to welcome you to the ENCALS Meeting 2017, taking place at the Cankarjev dom Cultural and Congress Centre in Ljubljana, Slovenia. The meeting is hosted by the Ljubljana ALS Centre and the Slovenian Society of Clinical Neurophysiology. In three days, a series of plenary lectures, thematic sessions and poster presentations will bring together international researchers and clinicians to discuss the advances in research and clinical care. As always the meeting is also an excellent opportunity to meet colleagues and friends and to start new collaborations.

Besides the main programme, there are numerous satellite meetings and events, including the 8th Annual Scientific Meeting of the Thierry Latran Foundation and the 3rd TRICALS Workshop Outcome Measures. I would also like to welcome the participants of the ALS Health Practitioners Forum, the first inaugural meeting of nurses, speech therapists, physiotherapists and occupational therapists providing ALS care.

I would like to thank Prof. Orla Hardiman, Prof. Leonard van den Berg and all the members of the Programme and Organising Committees for their input and support, Mrs. Akke Albada from ENCALS Office and Mrs. Alenka Kregar from Cankarjev dom Congress and Events Management for turning many complications into a smoothly organised event, and all the sponsors for their generous contribution.

I can promise you that an interesting meeting programme will effectively keep you away from exploring our beautiful city. Which means that you will leave with a good reason to visit us again soon.

Dobrodošli v Ljubljani!

Blaž Koritnik

On behalf of the Local Organising Committee

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## Venue

Cankarjev dom, Cultural and Congress Centre

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## Acknowledgements

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# Scientific Programme

## Thursday, 18 May

12:00-13:30 **Registration and lunch** / Second Foyer

### LINHART HALL

**Chairs: Leonard van den Berg, Janez Zidar**

13:30-13:45 **Opening session**

13:45-14:15 **Dr. Janez Faganel memorial lecture:** Prof. Jernej Ule (London, UK)
*„Protein-RNA complexes & ALS: insights from iCLIP"*

14:15-15:00 **Thierry Latran Foundation lecture:**
**Dr. Jesús S. Mora** (Madrid, Spain), **Prof. Luis Barbeito** (Montevideo, Uruguay), **Prof. Olivier Hermine** (Paris, France)
*„Masitinib as an add-on therapy to riluzole is beneficial in the treatment of amyotrophic lateral sclerosis (ALS) with acceptable tolerability: results from a randomized controlled phase 3 trial"*

15:00-16:00 **Session I:** Therapy

**Joseph M. Palumbo**: Efficacy and safety of edaravone (MCI-186) for the treatment of amyotrophic lateral sclerosis (ALS): a 24-week extension

**Ammar Al-Chalabi**: ODM-109 (oral levosimendan): key placebo-controlled results from the phase 2 study in ALS patients with SVC between 60-90% predicted at screening

**Joseph M. Scarrott**: Bringing gene therapy based SOD1 silencing towards human trials: a highly efficacious, off-target free and biomarker supported strategy for fALS

**Franziska Bursch**: Intraspinal injection of human mesenchymal stromal cells in SOD1G93A ALS mice

16:00-16:30 **Coffee break**

**Chairs: Magdalena Kuzma, Markus Weber**

16:30-17:30 **Session 2:** Clinical & Epidemiology

**James P. K. Rooney**: Euro-MOTOR: a multi-centre population-based case-control study of metals and solvents exposure as risk factors for amyotrophic lateral sclerosis

**Alexander Sherman**: Global ALS/MND "big data" collaboration environment in a post-PRO-ACT era

**Nayana Gaur**: The time of the ALSFRS-R to decrease to 50% (D50) in a sigmoidal decay model sufficiently describes the complete disease course of ALS

**Fabrizio D'Ovidio**: The role of pre-morbid diabetes on developing amyotrophic lateral sclerosis

17:30-19:00 **Poster session I** / Second Foyer

# Friday, 19 May

## LINHART HALL

07:30-08:00 Coffee reception for AB Science satellite meeting

08:00-09:00 **AB Science satellite meeting:**
**Luis Barbeito** (Montevideo, Uruguay): Masitinib for the treatment of amyotrophic lateral sclerosis (ALS): preclinical overview

**Chairs: Adriano Chiò, Julian Grosskreutz**

09:00-09:45 **Invited lecture: Dr. Federica Agosta** (Milano, Italy)
*"Tracking ALS progression using neuroimaging"*

09:45-10:45 **Session 3:** Genetics A

**Chen Eitan**: Discovery of microRNA gene mutations in ALS patient genomes

**Matthieu Moisse**: Whole genome sequencing as tool to unravel rare variants associated with ALS survival

**Alex Freischmidt**: Serum microRNA-profiles indicate a role of Fragile-X-related proteins for ALS

**Rick van der Spek**: The Project MinE data browser: bringing whole-genome sequencing data in ALS to researchers and the public

10:45-11:15 **Coffee break**

**Chairs: Peter Andersen, Jochen Weishaupt**

11:15-12:00 **Invited lecture: Dr. Russell McLaughlin** (Dublin, Ireland)
*„The panorama of ALS genomics"*

12:00-13:00 **Session 4:** Genetics B

**Ahmad Al Khleifat**: Integrating copy-number analysis with structural-variation detection in 50 ALS patients with two extreme survival phenotypes

**Gijs H. P. Tazelaar**: ATXN1: Expanding the spectrum of polyglutamine repeats in ALS

**Alfredo Iacoangeli**: A high throughput gene, environment and epigenetics database and analysis system for international ALS research

**Monica Nizzardo:** MicroRNAs analysis of patient-derived iPSCs as molecular therapy for ALS

13:00-13:15 **Late breaking news**
**Albert Ludolph:** A placebo-controlled investigator initiated trial (IIT) to evaluate the efficacy, safety and tolerability of 1 mg rasagiline in patients with amyotrophic lateral sclerosis (ALS) receiving standard therapy (riluzole)

13:15-14:15 **Lunch** / Second Foyer

## LINHART HALL

**Chairs: Sharon Abrahams, Blaž Koritnik**

14:15-15:00 **Invited lecture: Dr. Thomas Bak** (Edinburgh, UK)
*„Cognitive and behavioural symptoms in ALS: why are they there and how to assess them?"*

15:00-16:00 **Session 5:** Cognition & Imaging

**Ratko Radakovic**: The brief Dimensional Apathy Scale (b-DAS): Mokken analysis and scale reduction

**Marta Pinto-Grau**: Assessing behaviour in ALS: the importance of using disease-specific tools

**Martin Gorges**: Hypothalamic atrophy correlates with onset of disease-defining symptoms in patients with ALS

**Giovanni Novi:** A PET/CT approach to spinal cord metabolism

16:00-16:30 **Coffee break**

**Chairs: Albert Ludolph, Luc Dupuis**

16:30-17:30 **Session 6:** Disease mechanisms A

**Gina Picchiarelli**: Role of FUS in post synaptic neuromuscular junction differentiation

**Doris Lou Demy**: Developing vertebrate models to highlight the functional relevance of Nefl and miRNAs in ALS pathogenesis

**Steven Boeynaems**: Phase separation of C9orf72 dipeptide repeats perturbs stress granule dynamics

**Tariq Afroz**: Dynamic polymerization of TDP-43 in health and disease

17:30-19:00 **Poster session 2** / Second Foyer

20:30 **ENCALS dinner** / Festival Hall

# Saturday, 20 May

## LINHART HALL

**Chairs: Ammar Al-Chalabi, Ludo van den Bosch**

09:00-09:15 **Poster award for PhD students**

09:15-09:30 **ENCALS Young Investigator Award**

09:30-10:15 **Invited lecture: Janine Kirby** (Sheffield, UK)
*„Gene Expression Profiling in ALS: Past, Present and Future"*

10:15-11:15 **Session 7:** Disease mechanisms B

**Hortense de Calbiac**: Deciphering the function and mechanisms of C9ORF72 in ALS

**Raphael Munoz-Ruiz**: Live imaging of RNA dynamics for genetic forms of amyotrophic lateral sclerosis (ALS) in zebrafish

**Jasna Brčić**: Two G-quadruplex structures adopted by oligonucleotide model of ALS and FTD linked GGGGCC repeats

**Veronica Ferrari**: The role of valosin containing protein (VCP) in the clearance of toxic misfolded protein aggregates in amyotrophic lateral sclerosis

11:15-11:45 **Coffee break**

**Chairs: Caterina Bendotti, Boris Rogelj**

11:45-12:45 **Session 8:** Disease mechanisms C

**Ana Bajc Česnik**: Intranuclear (G4C2)n RNA foci, transcribed from C9ORF72 hexanucleotide expansion mutation, form paraspeckle-like structures

**Yolanda Gibson**: C9orf72 interacts with coilin and influences Cajal body dynamics and splicing

**Bart Swinnen**: Direct RNA toxicity in a transient zebrafish model of C9orf72 ALS is abrogated by PURA and p62

**Stephanie Duguez**: Secretion of toxic exosomes by muscle cells of ALS patients: role in ALS pathogenesis

12:45-13:00 **Closing of the meeting**

# Satellite meetings and side meetings

## Wednesday, 17 May

### Room E6

09:00-12:00 Thierry Latran Foundation Scientific Advisory Board meeting

### E1 Hall

13:30-18:30 Satellite meeting:
8th Annual Scientific Meeting of the Thierry Latran Foundation
– by invitation only

### Second Foyer

18:30-20:00 Thierry Latran Foundation standing buffet
– by invitation only

## Thursday, 18 May

### E1 Hall

09:00-12:00 Satellite meeting:
8th Annual Scientific Meeting of the Thierry Latran Foundation

### Room E6

09:00-12:00 OnWebDuals Project meeting

### M1 Hall

19:00-20:00 Project MinE meeting

### Lili Novy Club

20:00-22:00 Cytokinetics Investigator Reception (closed meeting)

## Friday, 19 May

### Linhart Hall

08:00-09:00 AB Science satellite meeting

### M2 Hall

13:15-14:15 ENCALS Executive Board meeting

### E1,2 Hall

09:30-18:00 Satellite meeting:
ALS Health Care Practitioners Forum

### M1 Hall

16:00-16:30 Discussion session: New therapies in ALS clinic

### M1 Hall

17:30-18:30 Strength/ALS-CarE Project meeting

## Saturday, 20 May

### E1 Hall, E2 Hall, M1 Hall, M3,4 Hall

09:30-13:00 Satellite meeting:
TRICALS Workshop Outcome Measures (session for research nurses)

13:00-14:00 TRICALS lunch (for TRICALS centres)

14:00-19:00 Satellite meeting:
TRICALS Workshop Outcome Measures (for TRICALS centres)

20:00-23:00 TRICALS dinner (for TRICALS centres)

## Sunday, 21 May

### E1 Hall, E2 Hall, M1 Hall, M3,4 Hall

09:00-13:00 Satellite meeting:
TRICALS Workshop Outcome Measures (for TRICALS centres)

# Poster Session I

## Thursday, 17:30-19:00

## Biomarkers

### P1

TDP-43-based biomarker development in ALS

Emily Feneberg, Elizabeth Gray, David Gordon, Kevin Talbot, Martin R. Turner

### P3

Phosphorylated neurofilament heavy chains in blood as biomarker for ALS?

Maxim De Schaepdryver, Benjamin Gille, Victor Herbst, Britta Brix, Philip Van Damme, Andreas Jeromin, Koen Poesen

### P5

Proteomic analysis in postmortem neurological tissue of subjects with amyotrophic lateral sclerosis (ALS)

Marina Iridoy, Leyre Martínez, Victoria Zelaya, Enrique Santamaría, Joaquin Fernández-Irigoyen, Ivonne Jericó

### P7

Internal control genes validation for qPCR analysis in lymphocytes from patients with amyotrophic lateral sclerosis

Ewa Usarek, Beata Kaźmierczak, Beata Gajewska, Anna Barańczyk-Kuźma, Magdalena Kuźma-Kozakiewicz

### P9

Blood polyunsaturated fatty acid composition is a biomarker for amyotrophic lateral sclerosis

Laura Robelin, Alexandre Henriques, François Salachas, Hélène Blasco, Gabrielle Rudolf, Martine Bergaentzle, Marie-Céline Fleury, Christian Andres, Philippe Corcia, Eric Marchioni, Jean-Philippe Loeffler, Pierre-François Pradat, Jose-Luis Gonzalez De Aguilar

### P11

The synergistic effect of IL-2, IL-6, IL-10, IL-13 and eotaxin influence longevity in transgenic SODIG93A mice

Laura Moreno-Martínez, Ana C. Calvo, Miriam de la Torre, Janne M. Toivonen, Leticia Moreno-García, Nora Molina, Gabriela Atencia-Cibreiro, Pilar Zaragoza, Alberto García-Redondo, Rosario Osta

**P13**

Expression of macrophage scavenger receptor (MSR I) in patients with motor neuron disease

Beata Chełstowska, Beata Gajewska, Beata Kaźmierczak, Anna Barańczyk-Kuźma, Magdalena Kuźma-Kozakiewicz

**P15**

Genetic and constitutional factors are major contributors to substantia nigra hyperechogenicity in ALS and other neurodegenerative diseases

Juan F Vázquez-Costa, José I. Tembl, Victoria Fornés-Ferrer, Fernando Cardona, Lluis Morales-Caba, Gerardo Fortea, Jordi Pérez-Tur, Teresa Sevilla

**P17**

The TGFß- system – a critical factor in disease progression of amyotrophic lateral sclerosis (ALS)

Sebastian Peters, Eva Zitzelsperger, Sabrina Küspert, Sabine Klatt, Rosmarie Heydn, Siw Johannesen, Markus Otto, Jochen Weishaupt, Joachim Weis, Ludwig Aigner, Tim-Henrik Bruun, Ulrich Bogdahn

## Clinical

**P19**

Use of noninvasive ventilation in the treatment of ALS in Europe versus in the US: results of an international ALS specialist survey

Leonard van den Berg, Merit Cudkowicz, Mamede de Carvalho, Angela Genge, Orla Hardiman, Carlayne Jackson, Noah Lechtzin, Hiroshi Mitsumoto , Vincenzo Silani, Jinsy Andrews, Sarah Kulke, Stacy Rudnicki, Terry Heiman-Patterson

**P21**

Living wills for amyotrophic lateral sclerosis's disease patients: a psychological and integral health care perspective

Noemí Morales, Anna Camps, Laura Baget, Sandra Blavi

**P23**

Secular trends of ALS incidence in an Italian population-based register, 1995-2014: evidence for a birth cohort effect in women

Adriano Chiò, Gabriele Mora, Cristina Moglia Umberto Manera, Antonio Canosa, Stefania Cammarosano, Antonio Ilardi, Davide Bertuzzo Enrica Bersano, Paolo Cugnasco, Maurizio Grassano, Fabrizio Pisano, Letizia Mazzini, Andrea Calvo

**P25**

Dry mass slope is a predictive factor in amyotrophic lateral sclerosis

Franck Patin, Areej Alrabiah, Salah Eddine Bakkouche, Stéphane Bletran, Christian R. Andrès, Patrick Vourc'h, Hélène Blasco, Philippe Corcia

**P27**

The natural history of dysphagia in ALS: a population-based study

Cristina Moglia, Andrea Calvo, Antonio Canosa, Stefania Cammarosano, Antonio Ilardi, Fabrizio Pisano, Gabriele Mora, Enrica Bersano, Letizia Mazzini, Umberto Manera, Adriano Chiò

**P29**

Spatio-temporal assessment of the association between environmental exposures and the occurrence of amyotrophic lateral sclerosis (ALS)

Maria Barceló, Marc Saez, Andrés Paipa, José Luis Moreno, Mònica Povedano

**P31**

Advance directives and the decision-making process in an ALS Unit in Spain

Amparo Martínez, Juan F. Vázquez-Costa

**P33**

Exploring respiration and swallowing interaction by diaphragm motor evoked potentials in ALS patients

Nazan Şimşek Erdem, Ferda İlgen Uslu, Selen Bozkurt, Hilmi Uysal

**P35**

A diagnostic pathway in Polish patients with ALS

Krzysztof Nieporecki, Katarzyna Szacka, Magdalena Kuzma-Kozakiewicz

**P37**

Assessment of the functional state of ALS patients in relation to physical activity

Jan Sznajder, Magdalena Kuźma-Kozakiewicz

**P39**

ONWebDUALS: the European project funded by national agencies under the patronage of Joint Programme – Neurodegenerative Disease Research (JPND)

M. de Carvalho, P. M. Andersen, M. Gromicho, J. Grosskreutz, M. Kuźma-Kozakiewicz, S. Petri, M. Piotrkiewicz, T. Podsiadły-Marczykowska, B. Stubendorf, K. Szacka, H. Uysal

**P41**

Motor neuron disease: a clinical case with unexpected evolution

Costanza Bisordi, Elena Caldarazzo Ienco, Monica Fabbrini, Michela Rossi, Anna Rocchi, Lucia Chicco, Gabriele Siciliano

## Cognition

### P43

Age- and education-adjusted cut-off scores for the German parallel versions of the ECAS

Jürgen Keller, Lisa Hartmann, Helena E. A. Aho-Özhan, Cynthia Vazquez, Markus Loose, Sarah Schulenburg, Lisa Bauer, Amon Krimly, Ingo Uttner, Sharon Abrahams, Albert C. Ludolph, Dorothée Lulé

### P45

Slovenian version of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

Ana Štublar, Petra Prunk, Vita Štukovnik, David Gosar, Janez Zidar, Leja Dolenc Grošelj, Lea Leonardis, Blaž Koritnik

### P47

Premorbid neuropsychiatric disease in patients with motor neurone disease in Scotland

Danielle Leighton, Louise Manson, Caroline McHutchison, Laura Sherlock, Judith Newton, Laura Stephenson, Shuna Colville, Sharon Abrahams, Siddharthan Chandran, Suvankar Pal

### P49

The contribution of social cognition to social behaviour: cognitive predictors of behavioural change in ALS

Tom Burke, Marta Pinto-Grau, Katie Lonergan, Orla Hardiman, Niall Pender

## Genetics

### P51

ATXN1 intermediate-length polyQ expansions are associated with C9orf72 ALS

Serena Lattante, Maria Grazia Pomponi, Amelia Conte, Giuseppe Marangi, Giulia Bisogni, Agata Katia Patanella, Emiliana Meleo, Marcella Zollino, Mario Sabatelli

### P53

Novel UBQLN2 mutations linked to amyotrophic lateral sclerosis and spastic paraplegia through defective proteolysis

Elisa Teyssou, Laura Chartier, Roselina Lam, Maria-Del-Mar Amador, Géraldine Lautrette, Marie Nicol, Selma Machat, Sandra Da Barroca, Carine Moigneu, Mathilde Mairey, Thierry Larmonier, Safaa Saker, Christelle Dussert, Sylvie Forlani, Bertrand Fontaine, Danielle Seilhean, Delphine Bohl, Séverine Boillée, Philippe Couratier, François Salachas, Giovanni Stevanin, Stéphanie Millecamps

**P55**

TBCE mutations cause early-onset progressive encephalopathy with distal spinal muscular atrophy

Antonella Sferra, Gilbert Baillat, Teresa Rizza, Sabina Barresi, Elisabetta Flex, Giorgio Tasca, Adele D'Amico, Emanuele Bellacchio, Andrea Ciolfi, Viviana Caputo, Serena Cecchetti, Annalaura Torella, Ginevra Zanni, Daria Diodato, Emanuela Piermarini, Marcello Niceta, Antonietta Coppola, Enrico Tedeschi, Diego Martinelli, Carlo Dionisi-Vici, Vincenzo Nigro, Bruno Dallapiccola, Claudia Compagnucci, Marco Tartaglia, Georg Haase, Enrico Bertini

**P57**

New FIG4 gene mutation causing fast progressing ALS phenotype: a case report

Giorgia Querin, Virginia Bozzoni, Cinzia Bertolin, Ilaria Martinelli, Cinzia Gellera, Elena Pegoraro, Gianni Sorarù

**P59**

A retrospective analysis of the genotype-phenotype relationships in familial MND and MND-FTD within a South London population

James Bashford, Caroline Hitchen, Bradley Smith, Christopher Shaw

**P61**

Intermediate CAG repeats in the ATXN2 gene in patients with amyotrophic lateral sclerosis from a Brazilian Research Center

Jessica Ruivo Maximino, Joyce Meire Gilio, Frederico Mennucci de Haidar Jorge, Gerson Chadi

**P63**

SOD1, TDP-43, FUS/TLS and C9orf72 genes in Serbian ALS patients: long term survey

Dušan Keckarević, Milena Janković, Milica Gagić, Milica Keckarević Marković, Miljana Kecmanović, Ana Marjanović, Ivan V. Marjanović, Ivana Novaković, Zorica Stević

## Imaging

**P65**

Neuroimaging patterns along the ALS-FTD spectrum: a multiparametric imaging study

Eoin Finegan, Taha Omer, Siobhan Hutchinson, Mark Doherty, Alice Vajda, Russell McLaughlin, Niall Pender, Orla Hardiman, Peter Bede

**P67**

A 18FDG-PET study on ApoE genotype in ALS

Andrea Calvo, Antonio Canosa, Angelina Cistaro, Cristina, Cristina Moglia, Maura Brunetti, Barbara Iazzolino, Marco Pagani, Adriano Chiò

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Myelin imaging in amyotrophic lateral sclerosis: a comparison with multiple sclerosis using quantitative magnetisation transfer

Marwa Elamin, Elizabeth Emsley, Rebecca Broad, Nick Dowell, Matt Gabel, Jamie Campbell, Waqar Rashid, Nigel P. Leigh, Mara Cercignani

**P71**

The role of iron-related hypointensities on brain MRI as a biomarker in amyotrophic lateral sclerosis

Juan F. Vázquez-Costa, Miguel Mazón, Joan Carreras, David Hervás, Jordi Pérez-Tur, Luis Martí-Bonmatí, Teresa Sevilla

## Mechanisms of disease

**P73**

Synaptotagmin 13 protects motor neurons from degeneration in ALS

Monica Nizzardo, Federica Rizzo, Michela Taiana, Silvia Tamanini, Ilary Allodi, Julio Aguila Benitez, Jik Nijssen, Gianna Ulzi, Valentina Melzi, Roberto Del Bo, Nereo Bresolin, Giacomo Pietro Comi, Eva Hedlund, Stefania Corti

**P75**

Establishing a patient-derived organoid model for studying cortical thinning in ALS

Renata Vieira de Sá, Paul R. Ormel, Lynn van der Beek , Sandra Kling, Lot D. de Witte, Elly M. Hol, Leonard H. van den Berg, Jeroen R. Pasterkamp

**P77**

Stress granules formation upon condition of chronic stress in human ALS fibroblasts

Claudia Colombrita, Valentina Gumina, Annamaria Maraschi, Alberto Doretti, Cinzia Tiloca, Vincenzo Silani, Antonia Ratti

**P79**

Molecular characterization of mouse optineurin insufficiency models

Andrea Markovinović, Tereza Ljutić, Ivana Munitić

**P81**

Implication of peripheral macrophages in amyotrophic lateral sclerosis

Aude Chiot, Sakina Zaïdi, Charlène Iltis, Laure Bernard, Bertrand Calippe, Delphine Bohl, Stéphanie Millecamps, Christian S. Lobsiger, Séverine Boillée

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Mutant superoxide dismutase aggregates from human ALS spinal cord transmit templated aggregation and fatal ALS disease in mice

Elaheh Ekhtiari Bidhendi, Johan Bergh, Per Zetterström, Karin Forsberg, Bente Pakkenberg, Peter M. Andersen, Stefan L. Marklund, Thomas Brännström

**P85**

Axonal transcriptome of stem cell-derived motor neurons in health and ALS

Jik Nijssen, Julio Cesar Aguila, Rein Hoogstraaten, Shangli Cheng, Qiaolin Deng, Eva Hedlund

**P87**

Evaluating the interdependence of misfolded SOD1 species in ALS pathogenesis

Desseille Céline, Peyrard Sarah, Vande Velde Christine

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Translating ribosome affinity purification from C9orf72- ALS/FTD patient-derived iPS Motor Neurons

Chaitra Sathyaprakash, Jakub Scaber, Nidaa Ababneh, Ruxandra Dafinca, Kevin Talbot

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The capacity to maintain stress granule assembly is impaired by a preceding chronic stress – the "first hit" can sensitise neurons to the "second hit"

Tatyana Shelkovnikova, Haiyan An, Mikhail Kukharsky, Natalia Ninkina, Vladimir Buchman

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The homeoprotein Engrailed 1 in spinal motor neuron survival

Stephanie Vargas, Stéphane Nedelec, Alain Prochiantz, Kenneth L. Moya

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Glial cell morphology, intracellular SOD1 distribution and elemental composition in the brainstem and hippocampus of the transgenic rat model of ALS

Stefan Stamenković, Tanja Dučić, Vera Stamenković, Alexander Kranz, Pavle R. Andjus

**P97**

Development of a virally-induced TDP-43 in vivo model of ALS

Barbara Scherz, Vera Niederkofler, Nicole Taub, Robert Zimmermann, Birgit Hutter-Paier

**P99**

Investigating the modifying role of EphA4 forward signaling in amyotrophic lateral sclerosis

Laura Rué, Lies Schoonaert, Mieke Timmers, Ludo Van Den Bosch, Philip Van Damme, Robin Lemmens, Wim Robberecht

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Phenotypic screening of PrP-hFUS-WT3 mouse model

Eveliina Pollari, Elisabeth Rossaert, Tom Jaspers, Roman Vangoitsenhoven, Bart Van der Schueren, Carla Cirillo, Pieter Vanden Berghe, Philip Van Damme, Wim Robberecht, Ludo Van Den Bosch

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Dual role of MHCI pathway in the development and progression of ALS in mouse models

Giovanni Nardo, Maria Chiara Trolese, Mattia Verderio, Julio Aguila Benitez, Jik Nijssen, Laura Comley, Eugenio Erba, Nicolò Panini, Nilo Riva, Giorgia Dina, Angelo Quattrini, Staffan Cullheim, Eva Hedlund, Caterina Bendotti

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Molecular characterization of a TDP-43 loss of function endothelial phenotype

Katrin Strecker, Bettina Pitter, Miha Modic, Vincenzo Caprese, Sebastian Lewandowski, Alexander Hruscha, Stefan Bonn, Eloi Montanez, Bettina Schmid

**P107**

TDP43 fragments clearance in a muscle model of sporadic ALS

M. E. Cicardi, V. Crippa, P. Rusmini, R. Cristofani, V. Ferrari, G. Vezzoli, M. Meroni, M. Galbiati, B. Tedesco, A. Poletti

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Inwardly rectifying potassium channel Kir4.1 in microglial cell clusters in the hSODG93A rat model

Mina Perić, Danijela Bataveljić, Pavle R. Andjus

**P111**

Expression of ALS-linked TDP-43 c-terminal domain reduces β-adrenergic-mediated cAMP signalling in cultured astrocytes

Jelena Velebit, Sonja Prpar Mihevc, Boris Rogelj, Robert Zorec, Nina Vardjan

**P113**

Excitability and calcium homeostasis of mutant SOD1-D90A iPSC-derived motor neurons

Norman Kalmbach, Reto Eggenschwiler, Max Naujock, Zhangyou Zhu, Tobias Cantz, Florian Wegner, Susanne Petri

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Gene profiling of human iPSC-derived motor neurons from sporadic ALS patients reveals a participation of mitochondria in the autonomous mechanisms

Gerson Chadi, Jessica Ruivo Maximino, Frederico Mennucci de Haidar Jorge, Chrystian Junqueira Alves

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A novel human in vitro model of motor neuron disease (MND) uncovers individual patient response to antioxidant drugs

Sufana Al Mashhadi, Monika Myszczynska, Matthew Stopford, Richard Mead, Pamela J. Shaw, Laura Ferraiuolo

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NF-κB constitutively activated in astrocytes enhances microglia response and induces a biphasic effect on MNs performance during ALS disease course

Najwa Ouali Alami, Christine Schurr, Tobias Boeckers, Thomas Wirth, Albert Ludolph, Bernd Baumann, Francesco Roselli

### P121

Biomarkers of inflammation in long-term G-CSF treated ALS patients

S. Johannesen, S. Peters, S. Klatt, J. Grassinger, T-H. Bruun, I. Kobor, T. Kammermaier, O. Hsam, Th. Grimm, J. Rösl, A. Khomenko, D. Baldaranov, W. Herr, W. Schulte-Mattler, U. Bogdahn

### P123

Dysregulation of ROCK and ERK in SODIG93A mice: combinatorial ROCK/ERK-inhibition as possible therapeutic approach?

Nadine Thau-Habermann, Sarah Pederson, Niko Hensel, Julia Kauder, Peter Claus, Susanne Petri

### P125

An assessment of treatment guidelines, clinical practices, demographics, and progression of disease among individuals with ALS

Koji Takei, Manabu Hirai, Fumihiro Takahashi, Kikumi Tsuda, and Joseph M. Palumbo

### P127

Human mesenchymal stem cell therapy reduces neuroinflamation in earlier symptomatic stages of ALS in SODI-G93A mice

Gabriela B. Chiarotto, Mateus Vidigal de Castro, Adriana S. S. Duarte, Angela C. M. Luzo, Alexandre L. R. Oliveira

# Poster Session 2

## Friday, 17:30-19:00

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Vascular basement membrane proteins in plasma of ALS patients are associated with degree of cortical perfusion and age at sporadic disease onset

Sebastian A. Lewandowski, Julia Remnstål, Anna Häggmark, Mun-Gwan Hong, Peter Lönneberg, Koen Poesen, Koen Van Laere, Ulf Eriksson, Philip Van Damme, Peter Nilsson

### P4

Analysis of chitotriosidase activity in plasma and CSF as a candidate biomarker for amyotrophic lateral sclerosis

Leyre Martínez-Merino, Marina Iridoy, Blanca Acha, Miren Roldán, Adriana Rivero, Pilar Irún, Miguel Pocoví, Maite Mendioroz, Ivonne Jericó

### P6

Lipidomic signatures in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis

H. Blasco, C. Veyrat-Durebex, C. Bocca, F. Patin, P. Vourc'h, J. Kouassi Nzoughet, G. Lenaers, C. R. Andres, G. Simard, P. Corcia, P. Reynier

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TDP-43 function in nervous tissue is essential for physiological autophagy

Pascual Torres, Omar Ramírez-Núñez, Gisel Barés, Ana B. Granado-Serrano, Victòria Ayala, Jordi Boada, Laia Fontdevila, Monica Povedano, Daniel Sanchís , Reinald Pamplona, Isidro Ferrer, Manuel Portero-Otín

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Autoimmunity profiling of amyotrophic lateral sclerosis plasma

Jennie Olofsson, Julia Remnestål, Sebastian Lewandowski, Jan Veldink, Anna Månberg, Peter Nilsson

### P12

Small fiber neuropathy characterization in the SODIG93A ALS mouse model

M. A. Rubio, M. Herrando-Grabulosa, J. J. Vilches, X. Navarro

### P14

Protein array enabled profiling of autoantibody repertoires in ALS, ALS-FTD and FTD

Julia Remnestål, Jennie Olofsson, Anna Häggmark-Månberg, Sebastian Lewandowski, Peter Nilsson

**P16**

Low IDL-B and high LDL-I subfraction levels in serum of ALS patients

Jean-Baptiste Delaye, Franck Patin, Eric Piver, Clément Bruno, Marina Wasse, Patrick Vourc'h, Christian R. Andres, Philippe Corcia, Hélène Blasco

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Expression of carnitine/acylcarnitine translocase in patients with motor neuron disease

Beata Gajewska, Beata Chełstowska, Beata Kaźmierczak, Anna Barańczyk-Kuźma, Magdalena Kuźma-Kozakiewicz

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Multidisciplinary care improves survival of patients with ALS – evidence from the Ljubljana ALS Centre

Polona Klavžar, Blaž Koritnik, Lea Leonardis, Leja Dolenc Grošelj, Mojca Kirbiš, Stanka Ristić Kovačič, Polona Klinar, Dušan Dumić, Janez Zidar

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The phenotypical variability of ALS and its relationship with survival. A 25 year experience in Catalonia

Montserrat Alemany, Carlota Mendez del Barrio, Andres Paipa, Raul Domínguez, Abdel Assialiuoi, Mónica Povedano

**P24**

Infrastructure and resources for ALS research

Maya Bronfeld, Idit Ron, Shay Rishoni

**P26**

Relevance of the methodology for analysing results – the SNIP model in amyotrophic lateral sclerosis

Susana Pinto, Mamede de Carvalho

**P28**

Survival in amyotrophic lateral sclerosis and its dependency on slow and forced vital capacity

Susana Pinto, Mamede de Carvalho

**P30**

Existential decision-making in ALS. A comparison of legal and medical frameworks in Germany, Poland and Sweden

Christian Weber, Anna Lindblad, Gisela Badura-Lotter, Barbara Fijalkowska, Katarzyna Ciecwierska, Peter Andersen, Magdalena Kuźma-Kozakiewicz, Albert Ludolph, Tomasz Pasierski, Niels Lynöe, Dorothée Lulé

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Motor nerve biopsy in motor neuron disease and motor neuropathy: clinical, prognostic and therapeutic implications

Nilo Riva, Francesco Gentile, Federica Cerri, Yuri Falzone, Francesca Gallia, Paola Podini, Giorgia Dina, Sandro Iannaccone, Christian Lunetta, Massimo Corbo, Raffaella Fazio, Eduardo Nobile-Orazio, Giancarlo Comi, Angelo Quattrini

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In ALS patients hypoventilation and cough inefficacy correlate with functional performance decline on the 6MWT

E. De Mattia, G. Sannicolò, M. Gualandris, E. Maestri, A. Lizio, M. Iatomasi, S. Lupone, B. Garabelli, E. Mollar, V. A. Sansone, F. Rao, C. Lunetta

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End-of-life decisions in ALS: from physicians' perspective

Krzysztof Barc, Helena Aho-Özhan, Dorothee Lule, Magdalena Kuzma-Kozakiewicz

**P38**

Does wellbeing of patients with amyotrophic lateral sclerosis depend on their population of origin?

Katarzyna Ciećwierska, Anna Maksymowicz-Śliwińska, Krzysztof Nieporęcki, Beata Pilczuk, Natalia Szejko, Jan Sznajder, Dorothée Lulé, Peter M. Andersen, Albert C. Ludolph, Magdalena Kuźma-Kozakiewicz

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Amyotrophic lateral sclerosis domain ontology (DALSO) – first results

T. Podsiadły-Marczykowska, M. Kuźma-Kozakiewicz, Maria Piotrkiewicz

## Cognition

**P42**

A conceptual multi-modal cognitive model of affective social cognition (MASC) in amyotrophic lateral sclerosis

Tom Burke, Marta Pinto-Grau, Katie Lonergan, Emmet Costello, Orla Hardiman, Niall Pender

**P44**

Digit Span Forward, a brief and simple test useful for predicting prognosis in ALS

Lucia Catherine Greco, Andrea Lizio, Gabriella Rossi, Matteo Querzola, Valeria Ada Sansone, Christian Lunetta

**P46**

A secondary analysis of the Reading the Mind in the Eyes Test within ALS: the development of a rapid and reliable short form

Tom Burke, Marta Pinto-Grau, Katie Lonergan, Emmet Costello, Orla Hardiman, Niall Pender.

**P48**

Is there an association between attention-deficit/hyperactivity disorder (ADHD) and amyotrophic lateral sclerosis (ALS)?

Leonie Ebel, Susanne Petri, Martina de Zwaan

**P50**

Preliminary evaluation of hopelessness and depression in patients with amyotrophic lateral sclerosis undergoing a Mesenchymal Stem Cell clinical trial

Maiara Gonçalves Gedo, Mirian Akiko Furutani de Oliveira, Jessica Ruivo Maximino, Gerson Chadi

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Investigation of new generation sequencing technologies and variant calling methods on Whole Genome Sequencing and Miseq data of ALS patients

Alfredo Iacoangeli, Aleksey Shatunov, William Sproviero, Ahmad Al Khleifat, Ashly Jones, Stephen J. Newhouse, Richard Dobson, Ammar Al-Chalabi

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CHCHD10 variants in amyotrophic lateral sclerosis: where is the evidence?

Gijs Tazelaar, Wouter van Rheenen, Project MinE ALS Sequencing Consortium

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Unexpanded C9ORF72 alleles are not ALS risk factors

Kevin Mouzat, Jovana Kantar, Anne Polge, Hélène Blasco, Philippe Corcia, Philippe Couratier, Pierre Clavelou, Raul Juntas-Morales, Nicolas Pageot, Cedric Raoul, Serge Lumbroso, William Camu

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ALS in Turkey: recent insights from genetic studies

Ceren Tunca, Fulya Akçimen, Cemile Koçoğlu, Hamid Hamzeiy, Cemre Coşkun, Betül Uysal, Güneş Birdal, Aslı Gündoğdu Eken, Aslıhan Özoğuz, A. Nazlı Başak

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SMN2 gene copy number and promoter methylation as disease modifiers of childhood-onset spinal muscular atrophy

Miloš Brkušanin, Ana Kosać, Vladimir Jovanović, Jovan Pešović, Goran Brajušković, Vedrana Milić Rašić, Dušanka Savić-Pavićević

### P62

First report of angiogenin gene mutations in patients with amyotrophic lateral sclerosis in Serbia

Milena Janković, Ivan Marjanović, Ana Marjanović, Stojan Perić, Ivana Basta, Ivana Novaković, Zorica Stević

### P64

Genetic heterogeneity of amyotrophic lateral sclerosis in the Hungarian population

Kornélia Tripolszki, Dóra Nagy, Bernadett Csányi, Antonia Ratti, Cinzia Tiloca, Vincenzo Silani, József I. Engelhardt, Péter Klivényi, Nikoletta Nagy, Márta Széll

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Markus Loose, Michael Wagner, Jürgen Keller, Helena E. A. Aho-Özhan, Sarah Böhm, Ingo Uttner, Hans-Peter Müller, Martin Gorges, Jan Kassubek, Albert C. Ludolph, Dorothée Lulé

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Imaging profiles of ALS-FTD cohorts: looking beyond C9orf72

Eoin Finegan, Taha Omer, Siobhan Hutchinson, Mark Doherty, Alice Vajda, Russell McLaughlin, Niall Pender, Orla Hardiman, Peter Bede

### P70

Relationship between brain metabolism and cognitive/behavioral functioning in ALS

Joke De Vocht, Stefanie M. A. Willekens, Jordy Evens, Dorothy Gors, Donatienne Van Weehaeghe, Peter Claes, Koen Van Laere, Philip Van Damme

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Spinal cord multi-parametric MRI for survival prediction in ALS

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Modelization of intrinsic motor neuron defects in amyotrophic lateral sclerosis with human induced pluripotent stem cells

Cynthia Lefebvre, Carine Dalle, Boris Lamotte d'Incamps, Stéphane Blanchard, Guillaume Tournaire, Diana Toli, Charles Nicaise, François Salachas, Lucette Lacomblez, Christian Lobsiger, Stéphanie Millecamps, Séverine Boillée, Delphine Bohl

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Motor neurons derived from induced pluripotent stem cells of patients with mutations in TARDBP show axonal transport defects

Raheem Fazal, Laura Fumagalli, Wenting Guo, Wim Robberecht, Catherine Verfaillie, Ludo Van Den Bosch, Philip Van Damme

### P78

ALS patient-derived astrocytes: a high throughput model for drug screening

Matthew Stopford, Monika Myszczynska, Richard Mead, Laura Ferraiuolo

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Novel combinatorial screening identifies neurotrophic factors for selective classes of ALS-relevant motor neurons

Sébastien Schaller, Dorothée Buttigieg, Alysson Alory, Arnaud Jacquier, Marc Barad, Mark Merchant, David Gentien, Pierre de la Grange, Georg Haase

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Macrophage migration inhibitory factor as a potential therapeutic candidate for familial ALS

Adrian Israelson

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Muscle microRNAs: small RNA sequencing and differential expression in Slovenian patients and healthy age-matched controls

Anja Kovanda, Lea Leonardis, Janez Zidar, Blaž Koritnik, Leja Dolenc Grošelj, Stanka Ristič Kovačič, Tomaž Curk, Boris Rogelj

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Diana Wiesner, Lilla Tar, Birgit Linkus, Luc Dupuis, Philip C. Wong, Albert Ludolph, Francesco Roselli

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The fecal microbiome of early-stage ALS patients

David Brenner, Andreas Hiergeist, Carolin Adis, André Gessner, Albert C. Ludolph, Jochen H. Weishaupt

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Modeling FUS-ALS hallmark neuropathology using patient-specific iPSCs and iPSC-derived cortical neurons

Julia Japtok, Xenia Lojewski, Marcel Naumann, Moritz Klingenstein, Peter Reinhardt, Jared Sterneckert, Stefan Putz, Maria Demestre, Tobias M. Boeckers, Albert C. Ludolph, Stefan Liebau, Alexander Storch, Andreas Hermann

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Generation of gene knockouts and gene replacements in human cell lines for disease modelling: a cautionary note

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Understanding mechanisms of truncating mutations in the FUS gene

Tatyana Shelkovnikova, Haiyan An, Vladimir Buchman

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Sensory neuropathy in progressive motor neuronopathy (pmn) mice is associated with defects in microtubule polymerization and axonal transport

Michael K. Schäfer, Sarah Bellouze, Arnaud Jacquier, Sébastien Schaller, Laurence Richard, Stéphane Mathis, Jean-Michel Vallat, Georg Haase

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Interactions between TAU, FUS and TDP-43 in neurodegenerative diseases

Annis-Rayan Bourefis, Luc Dupuis, Valérie Buée, Edor Kabashi

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Profiling of basement membrane proteins in plasma and cerebrospinal fluid within ALS and frontotemporal dementia

Julia Remnestål, Sebastian Lewandowski, Jennie Olofsson, Caroline Graff, Magdalena Kuzma-Kozakiewicz, Albert C. Ludolph, Katrin Marcus, Philip Van Damme, Jan Veldink, John M. Ravits, Anna Månberg, Peter Nilsson

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Progranulin functions as a cathepsin D chaperone to stimulate axonal outgrowth in vivo

Sander Beel, Matthieu Moisse, Louis De Muynck, Wim Robberecht, Ludo Van Den Bosch, Paul Saftig, Philip Van Damme

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Unravelling the molecular mechanisms behind corticospinal motor neuron degeneration in ALS

Christine Marques, Mathieu Fischer, Thibaut Burg, Jelena Scekic-Zahirovic, Anaïs Streit, Céline Keime, Marco A. Mendoza, Caroline Rouaux

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Generation and analysis of ALS-associated hnRnp. A knockout zebrafish lines

Lara Jansen, Bettina Schmid

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Mitochondrial Calcium Uniporter expression with disease progression in the G93AhSOD1 mouse model

Vedrana Tadić, Adam Adam, Saikata Sengupta, Thanh-Tu Le, Silke Keiner, Madlen Guenther, Beatrice Stubendorff, Otto W. Witte, Julian Grosskreutz

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Clinical, pathological and molecular characterisation of C9orf72-ALS leads to identification of novel therapeutic targets

Johnathan Cooper-Knock, Adrian Higginbottom, Guillaume Hautbergue, Paul Heath, J. Robin Highley, Christopher J. McDermott, Paul G. Ince, Stephen B. Wharton, Janine Kirby, Pamela J. Shaw

### P118

TDP-43-nucleoporin connection: a novel player in ALS?

Omar Ramirez-Núñez, Ana Belen Granado-Serrano, Victoria Ayala, Jordi Boada, Pascual Torres, Mónica Povedano, Isidro Ferrer, Reinald Pamplona, Manuel Portero-Otin

## Therapy

### P120

Oral supplementation with the omega-3 docosahexaenoic acid (DHA) in patients with amyotrophic lateral sclerosis (ALS): a randomized, double-blind, placebo-controlled, pilot study

Elisabet Romero, Monica Povedano, M.Núria Virgili, Maria A. Barceló, Yolanda Martínez, Andrés Paipa, Ainhoa Tejado, Joan-Carles Domingo

### P122

A safety analysis of edaravone (MCI-186) during the first 6-cycles (24 weeks) of ALS therapy from 3 randomized double-blind placebo-controlled trials

Alexander Kalin, Kaoru Ishizaki, Elvia Medina-Paraiso, Alex Kim, Zhang Yannong, Takanori Saita, Masahiko Wasaki

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Pharmacokinetic profile of edaravone: a comparison between Japanese and Caucasian populations

Yoshinobu Nakamaru, Atsuhiro Kawaguchi, Shuji Kinoshita, Koji Takei, Joseph M. Palumbo

### P126

A pharmacometabolomics approach in ALS: proof of concept in a clinical trial of olesoxime

Hélène Blasco, Franck Patin, Amandine Descat, Guillaume Garçon, Philippe Corcia, Patrick Gelé, Timothée Lenglet, Vincent Meininger, David Devos, Jean François Gossens, Pierre-François Pradat

# Information for Speakers and Poster Presenters

## Poster presentations

Posters will be displayed in the Foyer II throughout the congress. Display boards will be numbered. During the poster session, the presenting authors are kindly asked to be present next to their poster.

## Speakers' Centre

The technical organizer will give you additional instructions concerning your session and the presentation of your paper in the Speakers' Centre. The Congress staff will ensure that your presentation is downloaded on the computer in your designated session room. Please make sure that your computer presentation is fully operational before your talk. Only Power Point presentations on USB keys and portable hard disks will be accepted. Version MS PowerPoint 2010 is recommended. We suggest that your computer presentation is installed and tested at least two hours before your talk. A technician and a room attendant will provide assistance when needed.

The Speakers' Centre will have the same opening hours as the registration desk.

## Internet

Wireless internet connection is available in Foyer II. The name of the network is CD_GUEST. No login or password is needed.

# Registration and Fees

## Registration fee:

- Established researchers 245 EUR
- PhD students 175 EUR

## Registration (for both established researchers and PhD students) includes:

- Participation at all ENCALS lectures and access to the exhibition area
- Congress bag including programme booklet
- ENCALS Dinner on Friday evening
- Lunch at the exhibition area on Thursday and Friday
- Coffee at the exhibition area during the breaks

### Registration and Information Desk

The ENCALS Meeting Registration Desk, located in Foyer II of Cankarjev dom, will open as follows:

| | |
|---|---|
| Thursday, 18 May | 11:00–18:00 |
| Friday, 19 May | 8:00–19:00 |
| Saturday, 20 May | 8:00–13:00 |

## Social Programme

### Friday, 19 May 2017

20:00–22:30

**ENCALS Dinner** / Festival Hall (Vilharjeva street 11)

Included in the fee for regular participants.

Additional tickets: 50 EUR / person

Meeting point:

19:30 at Cankarjev dom, Erjavčeva Street (by bus) and return at 22:30

## General Information

### Conference Identification Badge

A conference identification badge will be included in the conference material provided upon registration. There will be no admittance to the Scientific Sessions without the conference badge. Invitations to social events will be collected at the entrance.

### Attendance Certificate

A Certificate of Attendance will be issued to all registered participants.

### Coffee Breaks

During breaks, refreshments will be served free of charge to participants wearing congress badges.

### Lunches

Working lunches (standing buffet) are included in the registration fee and will be served at lunchtime in Foyer II.

Meeting 2017

ENCALS

European Network to Cure ALS
18 - 20 May, Ljubljana, Slovenia

# Abstracts

## Protein-RNA complexes & ALS: insights from iCLIP

Martina Hallegger (1), Miha Modic (1, 2), Lilach Soreq (1), Ina Huppertz (1, 3), Nejc Haberman (1), Julian Zagalak (1), Rickie Patani (1), Jernej Ule (1)

1. The Francis Crick Institute and the Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK
2. Institute of Stem Cell Research, Helmholtz Zentrum München, Neuherberg, Germany
3. European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Mutations in several RNA binding proteins (RBPs) cause ALS, including TDP43, hnRNPA1, hnRNPA2/B1, FUS and MATRIN3. Disease-causing mutations are most often concentrated within the intrinsically disordered regions (IDRs) of these RBPs. To understand the mechanisms of these mutations, we and others study how these RBPs assemble into larger protein-RNA complexes. One approach we take towards this purpose is to study their protein-RNA interactions with the use of individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP), RNA-RNA interactions with Mass Spectrometry, and their function with RNA-Seq and PolyA-Seq. Our study indicates that the IDRs serve as a docking platform for protein-protein interactions, which in turn affect the RNA binding properties of RBPs.
Since ALS and all neurodegenerative diseases are linked to aging, we also wish to understand how their causative mechanisms are linked to the interplay of molecular events and cellular changes that take place upon aging in different brain regions. For this purpose, we characterized aging-altered gene expression changes across 10 human brain regions from 480 individuals ranging in age from 16 to 106. We found that astrocyte and oligodendrocyte-specific, but not neuron-specific genes shift their regional expression patterns upon aging, particularly in the hippocampus and substantia nigra, while the expression of microglia and endothelial-specific genes increase in all brain regions. In line with these changes, high-resolution immunohistochemistry demonstrated decreased numbers of oligodendrocytes and of neuronal subpopulations in the aging brain cortex, and glial-specific genes predict age with greater precision than neuron-specific genes. I will discuss the role that protein-RNA complexes may play in regulating cellular fates, and in neuron-glia interactions in aging and late-life diseases.

## Masitinib as an add-on therapy to riluzole is beneficial in the treatment of amyotrophic lateral sclerosis (ALS) with acceptable tolerability: results from a randomized controlled phase 3 trial

Jesús S. Mora (1), Luis Barbeito (2), Olivier Hermine (3, 4)

1. ALS Unit, Department of Neurology, Hospital Carlos III, Madrid, Spain
2. Institut Pasteur de Montevideo, Uruguay
3. Department of Hematology, Necker Hospital, University of Paris Descartes, France
4. AB Science, Paris, France

Objective: Evaluate masitinib plus riluzole (100 mg/kg) in amyotrophic lateral sclerosis (ALS).
Background: Masitinib, an oral tyrosine kinase inhibitor targeting CSF1R, generates neuroprotective effects by targeting aberrant microglial cells and regulating neuroinflammation.
Design: ALS patients received riluzole plus masitinib 3.0 mg/kg/day (M3.0), 4.5 mg/kg/day (M4.5), or placebo (1:1:1) up to 48 weeks. Two groups were defined according to pre-randomization ALSFRS-R progression: 'Normal Progressor' (NP) of <1.1 points/month, and 'Fast Progressor' of ≥1.1 points/month; thereby, reflecting the inherent heterogeneity of phenotype and disease progression within the overall population. Primary endpoint was absolute change in ALSFRS-$R_{[W0–W48]}$ (ΔALSFRS-R). The analysis was executed via a stepwise, sequential method with significance at P<0.05 (re-randomization). Step 1 was M4.5 in NP; step 2 was M3.0 in NP; step 3 was M4.5 in 'Normal+Fast Progressors' (NFP); step 4 was M3.0 in NFP. Missing data handled via LOCF with sensitivity analyses based on reason of discontinuation and single imputation methodology. Secondary endpoint analyses included progression free survival (PFS), progression being defined as ALSFRS-R deterioration of >9 points from baseline or death, quality-of-life by ALSAQ40, and FVC. Safety analysis included patients administered at least one dose of study drug.
Results: 394 patients (NFP cohort) were randomized to the M4.5 (130), M3.0 (131) and placebo (133) arms. The NP cohort comprised 330 patients: 106, 110 and 114, respectively. For the primary endpoint analysis (M4.5 in NP), masitinib showed a significant benefit in ΔALSFRS-R over placebo with a least-square means difference (ΔLSM) of 3.4 (9.2 vs.12.6); 95%CI 0.6–6.1; P=0.0158. In terms of ALSFRS-$R_{[W0–W48]}$ slope, masitinib showed a clinically meaningful retardation of 27% (0.77 vs.1.21 points/month). All sensitivity analyses were positive, with P<0.02 according to single imputation methodology. Masitinib also demonstrated benefit over placebo in the secondary variables, significantly improving median PFS by 25% (20 vs.16 months, P=0.0159); ALSAQ40 by 28.5% (ΔLSM of 19.4 vs.27.2, P=0.0078); and FVC by 22% (ΔLSM of 26.0 vs.33.4, P=0.0332). For M3.0 in NP, masitinib showed benefit over placebo for ΔALSFRS-R=2.73 (-8.6 vs.-11.3, a 24% improvement, P=0.0661); and FVC (ΔLSM of -23.1 vs.-27.9, a 17% improvement, P=0.1662) that did not reach statistical significance.

The benefit reached significance for ALSAQ40 (ΔLSM of 15.6 vs.23.7, a 34% improvement, P=0.0057). No significant difference was seen between treatment-arms for analysis according to the NPF cohort. Common (>10%) adverse events (AEs) with masitinib in the NP cohort were rash, nausea, diarrhea, and weight loss. Frequency of AEs, serious AEs, and severe AEs (placebo versus M4.5 and M3) was respectively: 79% vs. 90% and 84%; 20% vs. 28% and 18%; 18% vs. 24% and 17%.
Conclusions: Masitinib orally administered at 4.5 mg/kg/day as an add-on to riluzole demonstrated a significant therapeutic benefit with acceptable safety in ALS patients with a baseline ALSFRS-R progression rate of <1.1 points/month. Significant disease retardation was evident in terms of ΔALSFRS-R and ALSFRS-R slope (slowed loss of function), PFS (delayed progression), ALSAQ-40 (reduced decline in quality-of-life), and FVC (surrogate measure of survival). Masitinib may therefore be an important new therapeutic option for these patients.

## Tracking ALS progression using neuroimaging

Federica Agosta

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Current knowledge of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) is limited by poor understanding of how they progress through the central nervous system. In these patients, novel neuroimaging techniques may help to elucidate the spatial, time-dependent expansion of the underlying pathology across brain networks. Few magnetic resonance imaging (MRI) longitudinal studies have been published on ALS, and the true potential of MRI as a marker for monitoring disease progression has yet to be defined. Longitudinal analyses of ALS patients showed a decrease of cortical thickness in motor, temporal, and fronto-parietal cortices, as well as diffusion tensor MRI changes in the corticospinal tract, corpus callosum and frontal regions. The recent development and application of graph theoretical tools to MRI connectivity research offer a unique opportunity to explore principles of network-based neurodegeneration and to address unanswered questions. The application of graph theory on brain connectivity data put previous MRI findings in a new perspective, suggesting that node properties are likely to play a critical role in the pathophysiology of neurodegenerative diseases. Moreover, a major strength of graph theory is that it can be used to generate and test competing generative models designed to explain observed variations across a range of topological properties in the disease. Network science experiments will pave the way to the development of novel tools for understanding the biological underpinnings of ALS, and to identifying individualized, early interventions to modify disease progression.

## The panorama of ALS genomics

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ALS is a complex disorder, with no single genetic or environmental cause and no known cure. While heritability estimates show us that genetic risk factors clearly play an important role in the disease, epidemiological studies indicate that multiple separate accumulated risks over the lifetime of a patient also participate in triggering the disease process. However, identifying and understanding the nature of and interaction between these various genetic and (probable) environmental factors has been a challenge, and this incomplete understanding has been the greatest obstacle to the development of effective therapies for ALS. Technological advances in the field of genomics have accelerated the discovery of genetic risk factors for ALS; such studies have often been vast in scope, scale and cost. For example, genome-wide association studies (GWAS) involving up to 36,000+ individuals have identified novel risk loci and indicated a polygenic component to ALS. Further analysis of this polygenic risk has revealed a genetic correlation between ALS and schizophrenia, indicating shared genetic risk factors and potentially overlapping biology. However, the overarching conclusion from GWAS is that ALS has a principally rare variant architecture, the elucidation of which requires more fine-grained profiling and analysis of ALS genomes. To this end, a number of recent studies have identified genes that harbour rare ALS-causing mutations by sequencing the exomes (the entire protein-coding portion of the genome) of ALS patients. Ongoing work by the Project MinE Consortium is extending this approach to interrogate all rare genetic variation present across the entire genome. As discoveries continue to be made, a holistic view of ALS genomics will permit more effective patient categorisation and the identification of central disease mechanisms that reconcile the apparently multifarious genetic findings. This talk will explore the advances made in ALS genomics and discuss the successes and shortcomings of the approaches and technologies that have been adopted to date. Future avenues in ALS research will be discussed in light of a synthesis of evidence to date and the opportunities afforded by recent technological advances.

## Cognitive and behavioural symptoms in ALS: why are they there and how to assess them?

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For more than a century, our understanding of ALS has been characterised by a tension between the concept of a purely motor degeneration and a growing realisation of the frequency and importance of cognitive symptoms that can culminate in dementia. The increasingly frequent reports of cognitive and behavioural symptoms in ALS patients seemed to contradict the very idea of a "motor neurone disease". In my talk I will argue that cognitive and behavioural symptoms in ALS are a necessary and logical consequence of the motor character of the disease, as long as we extend our concept of the motor symptoms to encompass what I will call "motor cognition": the aspects of cognition most closely related to planning, control and understanding of movement. Apart from offering an integrated concept of the disease such an approach underlines the importance of cognitive assessment as part of the routine examination in ALS. In practical terms, brief but focused assessment tools, such as the Edinburgh Cognitive ALS Screen (ECAS) allow a meaningful and accurate bedside assessment of cognitive functions in ALS patients.

## Gene expression profiling in ALS: past, present and future

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Gene expression profiling has come a long way since the term was first widely used in the mid to late 1990's. With the advent of microarrays allowing the expression levels of increasing numbers of genes to be quantified, gene expression profiling, microarray analysis and transcriptomics have become frequently used terms associated with sampling RNA from multiple different tissue types. The results of these studies have been used to investigate disease mechanisms, monitor progression of disease and identify specific genes or gene signatures which could be used as diagnostic or prognostic biomarkers. Within the field of ALS, researchers have moved from using pooled spinal cord homogenates, to ensure they had sufficient material, through to isolating individual cell types from post-mortem cases and utilising peripheral tissues to determine biomarkers of disease and progression or stratification of patients.
Gene expression profiling has been a key methodology used in Sheffield and we initially used it to investigate disease mechanisms associated with mutant SOD1.
We were the first to demonstrate the disruption of the NRF2 signalling pathway in a cellular model of SOD1-related ALS, thereby establishing this as a potential therapeutic target which others demonstrated was more widely applicable to other forms of ALS. Several groups, including ourselves have now identified drugs targeting this pathway and it remains a viable target for therapeutic intervention.
As knowledge of the genome and transcriptome increased, microarrays evolved to sample not only gene-level expression but that of individual exons, allowing alternatively spliced transcripts to be detected; a timely development given the emerging role of RNA metabolism as a key pathogenic mechanism in ALS. We have demonstrated the disruption of splicing in TARDBP and C9orf72-related ALS, as well as in sporadic ALS cases. Transcriptomics has also expanded from sampling protein coding genes to non-coding transcripts such as miRNAs, which have the potential not only to act as more stable biomarkers, but also to regulate translation, and in themselves be used a therapeutic agents against specific targets.
With RNA-sequencing now becoming more affordable and with the advantages of identifying novel transcripts and detecting a greater range of expression levels, the future for gene expression profiling includes focusing on transcripts in specific cellular compartments, being an integral part of clinical trials and perhaps even a standard part of diagnostic procedures and therapeutic decisions.

# Masitinib for the treatment of amyotrophic lateral sclerosis (ALS): preclinical overview

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Masitinib is a selective oral tyrosine kinase inhibitor targeting CSF1R and c-Kit. Clinical data from the phase 3 study (AB10015) of masitinib in amyotrophic lateral sclerosis (ALS) demonstrated a clinically meaningful retardation of disease progression on its primary endpoint (change from baseline to week 48 in ALSFRS-R). Findings showed that masitinib administered at 4.5 mg/kg/day as an add-on to riluzole generated a therapeutic benefit with acceptable toxicity in ALS patients experiencing ALSFRS-R progression of <1.1 points/month at baseline. Significant slowing of disease was evident in terms of:

- ΔALSFRS-R and ALSFRS-R slope, indicating a slowed loss of function;
- Time elapsed between treatment initiation and ΔALSFRS-R of nine points (PFS), indicating delayed disease progression;
- ALSAQ-40 score, indicating reduced decline in quality-of-life; and
- FVC, which is considered a surrogate measure of overall survival.

The positive benefit–risk balance of study AB10015 signals that masitinib could provide an important new therapeutic option in this difficult to treat population. These positive clinical findings are supported by equally compelling preclinical data, showing masitinib to generate a neuroprotective effect through targeting aberrant microglial cells and regulating neuroinflammation.

It is well-established in the literature that proliferation and accumulation of microglial cells (microgliosis), in particular the emergence of aberrant glial cells, is a major neuropathological feature for ALS animal models. This disease mechanism is regulated by the CSF1/CSF1R signaling pathway, making microglia a viable target for masitinib. We have previously reported that masitinib treatment of $SOD1^{G93A}$ rats, initiated 7 days after paralysis onset (therapeutic setting), generated a robust protective effect as evidenced by a significantly prolonged post-paralysis survival with respect to control (40% improvement, $p<0.001$).

Immunohistochemistry data additionally showed that masitinib treatment: prevented microglia proliferation, migration and transformation into aberrant glial cells; reduced the number of aberrant glial cells in the degenerating spinal cord; improved microgliosis and motor neuron pathology; inhibited emergence of microglia proinflammatory phenotype; and inhibited microgliosis along the degenerating spinal cord.

Emergent data show masitinib is also capable of producing protective effects in the peripheral nervous system of $SOD1^{G93A}$ rats after paralysis onset. Strong upregulation of CSF1 and IL-34 in the degenerating sciatic nerve was observed, as well as a high infiltration of macrophages and mast cells. Masitinib treatment resulted in reduced pathological changes in the sciatic nerve, with a sharp decrease of inflammatory infiltrates of CSF1R-expressing macrophages and c-Kit expressing mast cells. Moreover, masitinib was seen to ameliorate neuromuscular pathology during paralysis progression in the ALS rat model, preventing mast cell accumulation around degenerative motor nerve endings, delaying endplate denervation as well as the pathological remodeling of neuromuscular junctions, perisynaptic Schwann cells and mu1scular capillary networks.

Considering the persistent failure of candidate ALS drugs over the past 20 years, the abovementioned clinical and preclinical data for masitinib mechanisms of action in both the central and peripheral nervous system, signal a genuine breakthrough.

## A placebo-controlled investigator initiated trial (IIT) to evaluate the efficacy, safety and tolerability of 1 mg rasagiline in patients with amyotrophic lateral sclerosis (ALS) receiving standard therapy (riluzole)

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Objective: The purpose of this trial was to assess the efficacy of rasagiline (1mg/day) as add-on therapy to the standard therapy with riluzole in patients with ALS compared to placebo. The primary objective was to investigate the survival time between both treatment groups. Secondary objectives included the change of total score of ALS Functional Rating Scale – Revised (ALSFRS-R), change in individual quality of life (SEIQoL, Schedule for the Evaluation of Individual Quality of Life), change of the slow vital capacity (sVC), time to tracheostomy or death, and safety parameters.

Background: The investigational drug tested in this trial (rasagiline) is a selective irreversible second-generation monoaminooxidase (MAO)-B inhibitor which showed neuroprotective effects both in in vitro and in vivo models for ALS, and dose-dependent effects on motor function and survival in mouse-model.
Design/Methods: This study was a double-blind, placebo controlled investigator initiated trial (IIT) of rasagiline in ALS patients. For entry in the study, the El Escorial Criteria for the diagnosis of ALS were used, and following main inclusion criteria had to be fulfilled: disease duration > 6 months and < 3 years, treatment with riluzole (100 mg/d) > 4 weeks, slow vital capacity (SVC)>50% of predicted normal, onset of progressive weakness within 36 months prior to study. 251 patients were randomly assigned to treatment with either placebo or 1 mg/d rasagiline according to their stratum (bulbar onset vs. spinal onset of disease, and study site). During a study treatment of 18 months, endpoint parameters were collected during 10 study visits. This IIT was conducted at 15 study centers of the German Network for Motor Neuron Diseases (MND-NET) with financial support of TEVA Pharmaceuticals Inc.
Results: The primary efficacy analysis showed no evidence that rasagiline as add-on therapy to riluzole prolongs the survival in patients with ALS. Subgroup analyses of individual strati (bulbar onset of disease, spinal onset of disease) did not show any significant differences between both treatment groups.
Analysis of secondary efficacy variables (functional: ALSFRS-R, lung function sVC) showed no difference between both treatment group. The analysis of quality of life (SEIQoL) showed nearly constant values over time and similar results for both groups. The time to tracheostomy or death showed no difference between both groups.
Safety analyses showed that rasagiline group and placebo group were comparable regarding the frequency of all Adverse Events including Serious Adverse Events (SAEs), drug-related SAEs and deaths under treatment.
Conclusions: The results of this Phase IIb study provide no evidence for a beneficial effect in patients with ALS. Rasagiline was well tolerated, both treatment groups were comparable regarding laboratory safety variables, and unexpected serious adverse events were not reported.

## Efficacy and safety of edaravone (MCI-186) for the treatment of amyotrophic lateral sclerosis (ALS): a 24-week extension

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Background: These are the results of a 24-week open-label (OL) extension period after a 24-week Phase III study. In the initial 24-week double-blind period (DB), edaravone was associated with less functional loss and quality of life deterioration than placebo (NCT01492686).
Objectives: To assess the efficacy and safety of an additional 24 weeks of edavorone (E-E) versus placebo switched to edaravone (P-E) in ALS patients.
Methods: Patients entered DB with retained broad functionality (all ALSFRS-R individual item scores ≥2 points) and normal respiratory function (%FVC ≥80%) at baseline. DB completers could enter OL to receive edaravone. OL was an additional 6 cycles of 60-mg intravenous edaravone once-daily for 10 of 14 days, followed by a 14-day drug-free period (28 days per cycle). The main efficacy endpoint was ALSFRS-R change across 12 cycles.
Results: Of the 137 patients who entered 24-week DB, 123 patients entered 24-week OL; 65/123 (E-E) and 58/ 123 patients (P-E). In 24-week DB, edaravone showed less decrease in ALSFRS-R compared with placebo (between-group difference 2.49 ± 0.76 at 24 weeks, p=0.001). In 24-week OL, E-E showed less observed change from study baseline in ALSFRS-R than P-E (between-group difference 4.17 ± 1.40 at 48 weeks). Edaravone improved ALSAQ-40, with a difference between edaravone and placebo in 24-week DB (8.79 ± 4.03, p=0.031), which was maintained in 24-week OL (10.71 ±4.51 at 48 weeks). Death or an event of certain disease progression occurred in 10 patients in E-E and 19 patients in P-E. The most commonly reported AEs (≥5% of patients in both treatment groups) were nasopharyngitis, respiratory disorders, constipation, dysphagia, and contusion.
Discussion: Edaravone demonstrated less functional loss than placebo at the 24-week endpoint. During the next 24 weeks, observed differences continued to be maintained; the E-E group appeared to show less functional loss than the P-E group at 48 weeks. The P-E group did not appear to ''catch up'' with the E-E group.
Conclusion: These observed findings suggest that early intervention with edaravone may confer a measureable clinical benefit when promptly initiated as opposed to being delayed by 6 months (in ALS patients who meet inclusion criteria and continue edaravone through one year).

## ODM-109 (oral levosimendan): key placebo-controlled results from the phase 2 study in ALS patients with SVC between 60-90% predicted at screening

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Levosimendan (i.v.) has been in clinical use for the acute worsening of severe chronic heart failure (CHF). In addition to cardiomyocytes, levosimendan sensitises also fast and slow skeletal muscle fibres to calcium by binding selectively to troponin C. Based on these findings, oral levosimendan (ODM-109) is now under development for symptomatic treatment of ALS. We report randomised, double-blind, placebo-controlled, 3-period, cross-over results of the LEVALS phase 2 study. The three study treatments were placebo and 1 mg and 2 mg daily doses of levosimendan. Each treatment period lasted for 14 days separated by wash-out periods. Primary objective of the study was to investigate the efficacy of oral levosimendan on respiratory function in patients with ALS. Primary efficacy variable was SVC% (sitting): There was a clear period effect in the data. Therefore period-wise baselines were justified in efficacy variable analyses (post-hoc). The estimated mean differences from baseline were 0.67, -0.98 and -0.01 % points for placebo, levosimendan 1 mg daily (p = 0.97 vs. placebo) and levosimendan 2 mg daily (p = 0.85 vs. placebo), respectively. For the secondary efficacy variable of SVC% (supine), the estimated mean differences from baseline were -3.62, +0.77 and +2.38 % points for placebo, levosimendan 1 mg daily (p = 0.018 vs. placebo) and levosimendan 2 mg daily (p = 0.001 vs. placebo), respectively. In other words, both levosimendan doses improved SVC% (supine) in a dose-dependent manner, whereas SVC% (supine) decreased during placebo. Both levosimendan doses were also numerically better compared to placebo in ALSFRS-R total score and respiratory domain scores. Levosimendan was generally well tolerated. The most common adverse events (AEs) reported as related to levosimendan were headache (due to vasodilatation) and increase in heart rate, both of which showed a dose-dependent increase in frequency. While most of the premature discontinuations without subjective symptoms were due the study medication stopping rule related to an increase in mean heart rate in 24-hour Holter recording, headache led to discontinuation in one subject. The number of severe AEs, serious AEs (SAEs) and supraventricular and ventricular tachyarrhythmias were similar between placebo and levosimendan treatments.

## Bringing gene therapy based SOD1 silencing towards human trials: A highly efficacious, off-target free and biomarker supported strategy for fALS

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Aims: 1. To evaluate the therapeutic efficacy of scAAV9-shRNA mediated SOD1 silencing in the SOD1-G93A mouse model using a clinic ready vector. 2. To investigate the measurement of CSF SOD1 protein levels as a biomarker of effective dosing and efficacy of SOD1 knockdown. 3. To investigate and evaluate miRNA-like sequence specific off-target effects on gene expression resulting from the viral delivery of a therapeutic shRNA targeting hSOD1.
Methods: Animals were treated at postnatal day 1 (P1, pre-onset) and P40 (onset). scAAV9-hSOD1si or scrambled control scAAV9-hSOD1ssi viral vectors were delivered via cisterna magna injection. Mice were tested using behavioural tests including weekly rotarod runs, neurological scoring and CatWalk gait analysis. Weekly body weight was also collected. Analysis of CSF by ELISA was used to determine SOD1 protein levels after treatment. Total RNA extracted from transduced isogenic Tet-inducible FLP-In HEK293T cell lines expressing either wildtype or G93A hSOD1 transgenes was analysed by whole genome microarray to elucidate potential off target effects.
Results: Cisterna magna injection of scAAV9-hSOD1si at P1and P40 improves motor performance and extends median survival in the mouse model by 42% and 13% respectively. Treatment at P1 also delays disease onset and significantly reduces SOD1 protein in the CSF as detected by ELISA, compared to scrambled controls. An in vitro assay using human cells demonstrated that the construct generated no off-target effects.
Conclusions: These findings are evidently important translational aspects of the study, in as much as: 1) a lack of observable off-target effects in human cells in vitro suggest the therapeutic shRNA construct is specific to hSOD1 and unlikely to elicit detrimental gene regulation in patients and 2) most clinical approaches suffer from lack of reliable biomarkers that correlate with treatment benefit. As the collection and analysis of CSF from patients is a relatively simple procedure, the measurement of SOD1 protein in CSF from patients undergoing SOD1 knockdown therapy has the potential to be a very useful and informative biomarker of therapeutic efficacy. Our approach therefore reinforces an already promising gene therapy strategy for clinical application in SOD1-linked familial ALS with additional translational aspects absent from previous studies.

## Intraspinal injection of human mesenchymal stromal cells in SOD1G93A ALS mice

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Introduction: Cellular therapy is being discussed as novel therapeutic option for the treatment of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS). Cell therapy for ALS currently focuses on the generation of a protective environment for motor neurons instead of cell replacement. Mesenchymal stromal cells (MSC) have already been shown to secrete different growth factors and have anti-apoptotic properties. They therefore appear suitable to create a neuroprotective microenvironment. As they can easily and safely be isolated from human bone marrow, MSC are promising candidates for further preclinical and clinical evaluation.
Methods: Human bone marrow derived mesenchymal stromal cells (hMSCs) are isolated by a previously established GMP-conform protocol. In the first study, SOD1G93A transgenic ALS mice (B6.SJL-Tg(SOD1-G93A)1Gur/J) receive intraspinal injections of either hMSCs (bilateral injections of 1x105 cells per side in a volume of 1µl as described), or saline as vehicle control before symptom onset (day 40). In a second study, mice are treated by intraspinal injections before and after symptom onset (day 40 and day 90). One group of animals receives saline on both days, one receives hMSCs at day 40 and saline at day 90 and the third group hMSCs at both days. Possible protective effects of hMSCs are evaluated by survival analysis, measurement of body weight and daily assessment of general condition according to a behavioral score. Motor performance is monitored via rotarod and footprint analysis.
Results/Conclusions: The results of our in vivo studies reveal a significant effect of hMSC injections at day 40 on weight loss and on step length (in male animals only) as well as a trend towards increased survival and improvement in general condition and motor performance. Preliminary results of the double injection study show significant effects of repeated intraspinal hMSC injections on motor performance (runtime analysis) and weight loss. Ongoing studies contain histological analyses of spinal cord tissue and further animal studies comparing intraspinal injection and intrathecal injections.

## Euro-MOTOR: a multi-centre population-based case-control study of metals and solvents exposure as risk factors for amyotrophic lateral sclerosis

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Introduction: Exposure to metals and solvents have been proposed as a risk factor for neurodegenerative disorders including amyotrophic lateral sclerosis (ALS), however research has been complicated by difficulty in assessing historical exposures.
Methods: Incident ALS cases and matched controls were recruited over 4 years in Ireland, Italy and the Netherlands. Trained investigators carried out structured interviews of participants to gather details of lifetime occupational history. Job-Exposure-Matrices (JEM) were applied to occupational data to characterize risk of exposure to pesticides. Logistic regression models adjusting for age, gender, education and cohort were used to determine the association between metal and solvent exposure and ALS risk.
Results: 1,557 patients and 2,922 controls were included. We found increased odds ratios (ORs) for ALS with any history of exposure to any airborne metals (OR = 1.25 95% CI: 1.05 – 1.49) and benzene (OR = 1.23 95%CI: 1.06 – 1.43). These findings were robust to sensitivity analyses, and were unchanged after correction for physical activity, smoking and alcohol consumption. Increased odds for exposure to chromium (OR = 1.29 95% CI: 1.02 – 1.64) and aromatic solvents (OR 1.17 95%CI: 1.00 – 1.36) were found only after adjustment for multiple confounders.
Discussion: Our findings provide new evidence for an association between metals and solvent exposure and ALS in European populations. Further work is ongoing to identify any population specific differences and exposure-response relationships.

## Global ALS/MND "big data" collaboration environment in a post-PRO-ACT era

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Platforms for global large-scale phenotyping allow investigators to collaboratively share clinical datasets, develop disease progression models and identify disease sub-populations and link clinical data to bio-, image-banks, WGS files, and omics to bringing ALS/MND into the Age of Precision Medicine and Big Data. While PRO-ACT database proved its efficiency as a de-facto reference set with tens of publications, several disease models and staging systems, its usefulness for disease modeling is somewhat limited. It is essential to identify new clinical datasets and utilize common approaches to data collection, integration, de-identification, sharing and distribution and incentives for Data Contributors. In NeuroBANK™, an accelerated patient-centric platform and environment for prospective clinical research, PALS' information is linked across studies, locations and modalities. CDEs, SOPs and Neurological Global Unique Patient Identifier (NeuroGUID) technology lead to accelerated studies' review, approval, deployment and enrollment, while preserving PALS' privacy. Standardized ICF language facilitates information sharing/data aggregation from multiple projects. Patient Portal allows integrating patient-reported outcomes (PROs) in Pooled Resource Open-Access Clinical Research (PRO-ACE) platform. Data from observational studies, retrospective clinical assessments, population registries and clinical datasets are eligible for inclusion of Data Use Agreements (DUAs). Defining data sharing terms are executed by Data Contributors who obtain ERB/IRB approvals and become members of PRO-ACE Consortium with benefits ranging from securing authorship to first-access rights. Cleaned, de-identified, harmonized datasets are merged into PRO-ACE Dataset available for open-access distribution for secondary analyses. International Data Access Committee reviews requests and grants access.

Results: NeuroBANK™ provides CDEs, data management, user training and certification, and help desk. 22 concurrent prospective research projects (6,000+ total enrollment goal), 200+ users from 94 research sites/17 countries capture data and track biofluids, tissues, cell lines, GWS files, images, omics and PROs in NeuroBANK™. Standard DUAs, MTAs, SOPs, and ICFs are implemented. NeuroBANK™/PRO-ACE data are devoid of placebo effects and clinical trial selection bias.
Conclusions: Available platforms and developed approaches allow for truly global collaboration.

## The time of the ALSFRS-R to decrease to 50% (D50) in a sigmoidal decay model sufficiently describes the complete disease course of ALS

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Introduction: Deterioration in ALS is not linear but this is what the routinely used progression rate [PR = (48-ALSFRS-R/disease duration)] suggests, as it describes progression at a circumscribed time point rather than over the disease course.
A model reflecting disease progression across time will facilitate theragnostic stratification of ALS patients based on severity and progression type and assist in drug development and clinical trial recruitment.
Objectives: The study aimed to develop a model that uses regularly collected ALSFRS-R scores to mathematically project disease course for individual patients.
Methods: The model enlists the observation that the ALSFRS-R decays slowly after symptom onset rather than dropping immediately. This transitions to a period of stable progression that has been captured by most clinical trials, owing to late inclusion of patients based on laboratory supported ALS as per the EL Escorial/ Awaji criteria. As disability progresses, ALSFRS-R appears to plateau again. Thus, we used a function which describes the transition between two states: full health to maximum disease. The model yields two parameters describing the ALS disease course: D50 = time taken for ALSFRS-R to drop to 24 and dx = slope of ALSFRS-R decrease. Capturing the disease coursebolsters the classification of disease phases: Phase I (early semi-stable phase), Phase II (early progressive phase), Phase III (late progressive phase) and Phase IV (late semi-stable phase).
Results: Based on ALSFRS-R scores and disease duration from onset to ALSFRS-R date for our cohort (n = 393) , we were able to determine D50 and dx in 352 (90%) of patients using the Microsoft® Excel Add-In Solver tool, with dynamic presets derived using the conventional PR parameter. Mean age at symptom onset was 62.8 years. ALSFRS-R was 36.9 +/- 7.8 and 26.3 +/- 10.6 at the first and last visit, respectively. The relationship between D50 and dx was highly linear (R2 = 0.9), indicating that the entire disease course can be described using D50 alone.
Conclusion: D50, defined as months taken to reach an ALSFRS-R of 24, is a more accessible and descriptive indice. Further, sampling at a given time point can be correlated to a single parameter, enabling the discovery of early prognostic markers and refining clinical endpoints.
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## The role of pre-morbid diabetes on developing amyotrophic lateral sclerosis

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Background: The literature on the association between diabetes and amyotrophic lateral sclerosis (ALS) produced contrasting results. This study was developed in order to assess the role of diabetes on the risk of developing ALS.
Methods: The study population was represented by all residents in Turin (Italy) at the beginning of 1996, already present at 1991 Census, older than 14 years (n=727,977), followed up for diabetes and ALS occurrence from 1998 to 2014. Presence of diabetes was ascertained through two Piedmont regional sources: the Diabetes Registry and the ATC Drugs Prescriptions Archive. The risk of ALS was estimated using the Piedmont and Valle d'Aosta ALS Registry (PARALS). The association of diabetes, treated as a time-dependent variable, with ALS onset was estimated through Cox proportional hazard regression models adjusted for age, gender, education and marital status:
Results: During the follow-up, 397 subjects developed ALS, 20 of whom were already diabetics before ALS onset. Diabetes identified through the Diabetes Registry or drugs prescriptions more than one year before ALS onset significantly decreased the risk of ALS (HR=0.26; 95% CI: 0.17-0.42).
Conclusions: The study results support a protective role of diabetes toward ALS.

## Discovery of microRNA gene mutations in ALS patient genomes

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MicroRNAs, endogenous small RNAs, have been suggested to play a role in many pathologies, including neurodegeneration. We recently demonstrated that Dicer activity is impaired in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease of the human motor neuron system, causing dysregulation of miRNA biogenesis. Accordingly, conditional loss of Dicer allele in mice is sufficient to cause motor neuron disease. Nowadays, we are directly testing the existence of causative mutations in microRNA genes in patients suffering from ALS. To this end, in collaboration with project MinE, we performed in silico capture, which calls specific genomic regions of interest from whole genome sequencing data. Our approach enabled us to capture all known microRNA genes, their protein cofactors and the 3' untranslated region of disease-associated genes. We then performed an extensive bioinformatics analysis to identify gene variants that are associated with disease, and further developed methodologies to weight the impact of the newly-identified mutations in miRNA genes. The analysis of miRNA gene mutations in ~4300 ALS patients, by in silico capture, is unprecedented. The data and approaches developed to reveal the centrality of noncoding RNA genetics in human disease will plausibly influence future genetics studies, beyond the specific pathology in focus.

## Whole genome sequencing as tool to unravel rare variants associated with ALS survival

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Background: Amyotrophic lateral sclerosis (ALS) usually leads to death within 3 to 5 years, but a high variability in patient survival has been observed, with 5% of the patients surviving more than 10 years (1). To date, several clinical factors have been associated with patient survival, e.g. gender, age at onset, site of onset, presence of frontotemporal dementia, … (2). Additionally, also genetic variants, like the C9orf72 repeat expansions, have been shown to associate with survival. Recent genome-wide association studies (GWAS) using common genetic variant could only reveal the association of a small number of loci with ALS survival, leaving a large number of cases genetically unexplained.
Objectives: To identify rare genetic variants that associated with ALS survival using whole genome sequencing (WGS) data.
Methods: We used WGS data from 1,577 Belgian and Dutch ALS patients, sequenced as part of Project MinE. Cox-regression was applied on a genome wide scale using the GenABEL-package, while correcting for age at onset, site of onset, gender, sequencing technology and the first 10 PCA's for population stratification.
Results: Cox regression analysis revealed 196 rare variants associated with ALS survival with a P-value between 5e-8 and 3.7e-13 and a minor allele frequency between 0.03% and 8.8%. The hazard ratio of the variants ranged from 0.5 to 3.8.
Discussion and conclusions: The fact that several rare variants associate with survival, underscores the importance to consider the effect of rare variants in ALS pathology, especially in survival. The discovery of these novel loci as survival modifiers in ALS is important, as they can lead to an improved understanding of the disease process, which is of crucial importance in the development of treatment options.

But due to the nature of rare variants, the incidence of the variants is low and caution should be taken with possible false positive finding, making validation in other cohorts desirable. The fact that these samples are part of the Project MinE will give us an ideal opportunity to validate our finding in a larger cohort.

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Acknowledgements: Samples were sequenced as part of the MinE project. Belgian samples were collected at UZ Leuven and Dutch samples at UMC Utrecht.

## Serum microRNA-profiles indicate a role of Fragile-X-related proteins for ALS

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In previous studies we identified a subset of downregulated serum microRNAs (miRNAs) in familial and a majority (~60%) of sporadic ALS patients that was already evident in pre-clinical mutation carriers. A common 5-nucleotide-sequence motif (GDCGG; D = G, A or U) was highly significantly enriched in the downregulated miRNAs indicating the deregulation/malfunction of one or several specific RNA-binding protein(s). Using miRNA-pulldown assays in lysates of HEK293 cells followed by mass spectrometric identification and quantification of precipitated proteins, we identified 37 proteins specifically associated with the GDCGG-motif. Direct and specific binding to miRNAs with the GDCGG-motif of two of the top candidate proteins, FXR1 and FXR2, was confirmed by biochemical assays. Binding domains could be mapped to the RGG-domain of FXR1 and the second RG-domain of FXR2. The third member of the Fragile-X-related protein family, FMRP, causes Fragile-X mental retardation when absent and has already been implicated in ALS pathology. Although not a top candidate protein in our miRNA-pulldown assays, we show direct and specific binding of FMRP to miRNAs with the GDCGG-motif via its RGG-domain highly homologous to the RGG/RG-domains of FXR1 and FXR2, respectively. In vitro binding studies on a transcriptome-wide scale using miRNA-arrays identified the member of the Fragile-X-related protein family most likely responsible for altered miRNA-profiles in ALS and expression analysis and neuropathological studies will be employed to validate its role in ALS pathogenesis.

## The Project MinE data browser: bringing whole-genome sequencing data in ALS to researchers and the public

Project MinE ALS sequencing consortium

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Project MinE is an international collaboration with the aim of whole-genome sequencing 15,000 amyotrophic lateral sclerosis (ALS) patients and 7,500 controls at ~30x coverage. The data generated by Project MinE currently comprises > 6,500 whole-genomes, and is highly relevant for ALS-specific research. Additionally, the data generated by Project MinE will provide both clinical and research geneticists with a large-scale resource. With the aim of making this resource publicly-accessible, we have created the Project MinE data browser (databrowser.projectmine.com), an open-access server containing currently available Project MinE data and summary statistics from previous published studies. The data browser, based on R shiny, offers fast and easy access to all genetic variation (both common and rare) observed in Project MinE, allele frequency information in cases and controls drawn from Europe and the US, genic association testing results, functional annotation, and integration with publically available gene expression profiles (GTEX). One can simply enter a gene and view depth of coverage, burdentests, gene expression profile and variant information in a single page. Through its visual components and interactive design, the browser specifically aims to help those without a biostatistics background to integrate Project MinE data into their own research. The browser will continue to grow as Project MinE does, and in the future will include integration with the Allen Human Brain Atlas, ExAC/gnomAD and Varsome.

## Integrating copy-number analysis with structural-variation detection in 50 ALS patients with two extreme survival phenotypes

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly of motor neurons, characterized by progressive weakness of voluntary muscles and death from respiratory failure due to diaphragmatic paralysis, typically within 3 years of onset. Despite the very poor prognosis, there is considerable variation in the survival rate, and up to 10% of people with ALS live more than 8 years from first symptoms. There is a strong genetic contribution to ALS risk. In 5% of cases or more, a family history of ALS or frontotemporal dementia is obtained, and the Mendelian genes responsible for ALS in such families have now been identified in about 70% of cases. Even in apparently sporadic cases, twin and population studies show the heritability is about 60%. Although risk genes reveal information about the mechanism of causation of ALS, it is also important to identify gene variants that modify survival. Survival genes could potentially be targeted directly, or their product augmented to improve ALS survival. A number of common gene variants associated with ALS survival have been identified through genome-wide association studies or other genome-wide approaches like studying structural variants.
Aim: Is to know the structural variations difference between different ALS survival groups.
Methods: Analysis of copy-number variation using whole-genome sequencing data of 50 ALS patients with two extreme phenotypes, 25 short lived patients against 25 long survived ALS patients using Copy Number Segmentation by Regression Tree in Next Generation Sequencing (CONSERTING).
Samples: The top and bottom 1.5% of ALS patients by survival were identified (25 patients from each tail of the distribution). All patients were classified as definite or probable ALS according to the El Escorial criteria and had no family history of ALS. Sample ancestry and relatedness were evaluated by principal components analysis and relationship matrices.

## ATXN1: expanding the spectrum of polyglutamine repeats in ALS

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Objective: Polyglutamine proteins can cause a wide range of neurodegenerative disorders upon long-range expansions such as Huntington's disease and spinocerebellar ataxia (SCA). Intermediate length of the polyglutamine coding CAG/CAA trinucleotide repeat in the ATXN2 gene was shown to be a risk factor for amyotrophic lateral sclerosis (ALS). Involvement of other CAG-repeat genes has only been moderately investigated or reported, although an association was found with intermediate length in the ATXN1 in a small Italian cohort after discovery of a pedigree with co-occurrence of ALS and SCA1 disease. After identification of a similar pedigree, we set out to investigate the frequency of ATXN1 intermediate expansions in a large international cohort of ALS patients and controls. Methods: We screened the ATXN1 CAG/CAT trinucleotide repeat length via PCR in DNA of 2,742 ALS patients and 2,374 controls from 4 different countries (Belgium, France, Ireland and The Netherlands). PCR-fragment repeat size was additionally validated using Sanger sequencing in a subset (N=850) of the data. An intermediate cut-off was determined via control distribution and was found to be at 33 CAG/CAT repeats and higher.

Results: We found 242 (11.4%) intermediate expansion carriers in control individuals and 333 (13.8%) in ALS patients. Meta-analysis of the frequencies in the 4 different cohorts showed significantly more expanded repeats in ATXN1 in ALS patients compared to controls (p = 0.006, one sided) with an odds ratio of 1.25 (95% CI: 1.05-1.50).
Interpretation: Similar to ATXN2, there is an association with an increased length of CAG(/CAT) trinucleotide repeats in ATXN1 and ALS. Interestingly, in contrast to ATXN2, we found a relative high frequency of intermediate expansions in ATXN1 in both ALS (1-2% in ATXN2 vs 6-7% in ATXN1) and control individuals (0.3-0.4% in ATXN2 vs 5-6% in ATXN1). This fits with the hypothesis that intermediate polyglutamine expansions might not be causative but pose an increased risk for developing ALS. However, this overall risk in ATXN1 seems to be smaller compared to intermediate carriers in ATXN2 (OR 3.06 in ATXN2 vs OR 1.25 in ATXN1). Future experiments will focus on both the similarities between ATXN1 and ATXN2, such as TDP-43 binding capacity, as well as the differences, such as the CAT-interruptions in ATXN1, to further elucidate the role of polyglutamine expansions in ALS.

## A high throughput gene, environment and epigenetics database and analysis system for international ALS research

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Genetic technology is advancing rapidly. We now have the ability to quickly and cheaply collect huge amounts of genetic information, and because of close collaboration between research groups, we can do this with tens of thousands of people. The problem is how to store, handle and easily share this information. This project is a collaboration between researchers working on motor neuron disease, and computer scientists working with biological information. We aim to develop a computerized system that will let researchers easily use genetic, clinical and lifestyle information that has already been collected, and add new information as it is produced. The system will make it easy to see patterns in the relationship between clinical features, lifestyle, and gene variations, to compare genetic variations between groups of people, and to share the information between research groups. We are implementing a solution that will enable the sharing of huge raw sequencing data as well as small files for summary results. For raw and processed data we used iRODS, an integrated Rule-Oriented Data System, developed to build distributed storage infrastructure. Through data virtualization several iRODS servers in different locations can share and manipulate their data through automatic mechanisms based on internal rules. This would facilitate sharing, curating and the analysis of the huge amount of data that our genetic research is producing. An iRODS system able to host and deal with petabytes of genetic data, has been deployed on Rosalind, our BRC/King's College London HPC cluster. Data is accessible both through a user friendly web browser and the command line. Results and summary statistics data, along with the clinical data, will be loaded into the TranSMART platform. The TranSMART system is a platform for translational medicine comprising a relational database back end and a web based interface that integrates a large number of open source bioinformatics tools for analysis and visualization. This platform will provide general user access to processed data and will allow for cohort selection and analyses on the fly. We are also implementing community driven metadata standards and pipelines for their extractions and data analysis which can be automatized using iRODS. All Pipelines will be available on github together with iRODS Docker images to allow any member of the ALS research community to quickly deploy their own iRODS timescale of hours.

## MicroRNAs analysis of patient-derived iPSCs as molecular therapy for ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive degeneration of motor neurons (MNs). The mechanisms underlying the disease and specific proteins involved are almost unknown, even if the dysregulation in RNA metabolism represents a major contributor to ALS aetiology. Mutations in genes encoding for DNA/RNA-binding proteins, such as TDP-43 and FUS, and the hexanucleotide intronic repeat expansions in C9ORF72 have been associated with familial ALS (fALS) and represent the first genetic cause of sporadic ALS (sALS). In particular, TDP-43 and FUS have been implicated in several steps of RNA metabolism, including microRNA (miRNA) processing. MiRNA are tissue-specific small molecules that can individually regulate several hundred targets by RNA-dependent mechanism. Since miRNAs are required for the survival of specific types of mature neurons in model organisms, they may play important roles in the aetiology or progression of neurodegenerative disorders. We and other groups have demonstrated that ALS-linked genes can affect miRNA expression. Here we aim to investigate the role of miRNAs dysregulation and their relative proteomic changes in induced pluripotent stem cells (iPSCs) derived from fALS/sALS patients, based on in vitro models developed in our lab. We performed Next Generation Sequencing (NGS) analysis on iPSC-derived motor neurons in order to identify dysregulated miRNAs in ALS and we further characterized them and their biological targets by bioinformatic tools, molecular and proteomic studies in vitro and in vivo. This approach can increase the chances of modifying complex diseases, such as ALS, by targeting the entire gene networks. Moreover, the identification of feasible miRNAs as targets of the disease can lead to the discovery of new disease biomarkers and therapeutic strategies.

## The brief Dimensional Apathy Scale (b-DAS): Mokken analysis and scale reduction

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Background: Apathy is a prominent demotivational symptom in neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). It is considered a multidimensional syndrome, composed of different subtypes which can be measured using the Dimensional Apathy Scale (DAS), a 24 item tool assessing Executive, Emotional and Initiation apathy, independent of motor disability. Due to increasing diagnosis, awareness and impact of apathy, a concise yet comprehensive measure is needed in clinic. The aim was to reduce the DAS using a large mixed neurodegenerative disease sample (AD and ALS) to form the brief DAS (b-DAS).
Method: Data from 102 non-demented ALS patients and 102 AD patients of responses to the informant/carer DAS were utilised, with additional availability of informant/carer Apathy Evaluation Scale (AES) and the Geriatric Depression Scale-Short form (GDS-15), standard apathy and depression measures. Mokken analysis was performed on each DAS subscale (Executive, Emotional and Initiation) for initial item reduction based on discrimination (*Hi*). Item endorsement (mean item score) was also examined. Item-total correlational analysis was performed, with the AES total, to determine convergent validity, and the GDS-15 total, to determine divergent validity, to establish the final structure of the b-DAS.
Results: AD and ALS patients were well matched for years of education, but differed on gender distribution and age. However, there was no correlation with age and no gender differences on apathy or depression. Mokken analysis on each DAS subscale resulted in all 8 Executive and all 8 Initiation items being retained, with 3 Emotional items showing a weak *Hi* (< .3) and were consequently removed. Of the remaining items, those with a stronger positive correlation with the AES ($r$ > .5) and also a moderate to weaker correlation with the GDS-15 ($r$ < .5), as well as those theoretically coherent to each subscale, were selected. This resulted in the b-DAS composed of 9 items, equally weighted over the Executive, Emotional and Initiation subscales.
Conclusion: The b-DAS is a robust yet short multidimensional apathy instrument composed of 9 items that correlate with a gold-standard apathy measure, whilst reducing the association with depression. It is appropriate for use in the clinic and research to quickly and comprehensively screen for apathy subtype impairments in neurodegenerative disease.

## Assessing behaviour in ALS: the importance of using disease-specific tools

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Background: Behavioural changes are a core aspect of Amyotrophic Lateral Sclerosis (ALS). General behavioural assessments, such as the Frontal Systems Behaviour Scale (FrSBe), have been widely used to assess behaviour in ALS, although these measures tend to overestimate the presence of changes as the influence of motor dysfunction on behaviour is not considered. ALS-specific behavioural scales correcting for such confounders have been developed. The Beaumont Behavioural Inventory (BBI) is a 41-item proxy-report questionnaire which assesses the whole spectrum of behaviours observed in ALS. The BBI has shown high internal consistency (Cronbach's alpha=0.891).
Objective: This work aims to compare two BBI validation studies, one using the FrSBe as the gold standard, and the other comparing it against another ALS-specific tool, the ALS-FTD-Q.
Methods: The BBI has been validated against the FrSBe in a sample of 85 ALS patients, and has also been compared to the ALS-FTD-Q in an additional sample of 60 patients.
Results: In both studies, the BBI demonstrated good construct validity. Highly significant positive correlations were observed between the BBI and the FrSBe ($r=0.760$, $p<.0001$), and the BBI and the ALS-FTD-Q ($r=.807$, $p<.0001$), indicating adequate convergent validity. In both studies, no correlations were observed between the BBI and non-behavioural measures, which indicated good discriminant validity. A cut-off score of ≥7 for the presence of behavioural changes was derived from an age-, gender-, and education-matched healthy control sample (n=78). When validated against the FrSBe, this cut-off showed high sensitivity (88%) and specificity (79%). When compared to the ALS-FTD-Q, high sensitivity was observed (100%), but specificity was decreased (70%). Further analysis of behavioural aspects endorsed by cases classified as 'false positives' showed that changes most frequently reported included behavioural aspects not measured by the ALS-FTD-Q, such as diminished social interest, excessive sensitivity to sensory stimuli, and the presence of grammatical mistakes.
Conclusions: General behavioural instruments that do not correct for motor disability tend to overestimate the presence of behavioural changes in ALS. Disease-specific instruments that do not include the whole range of behaviours characteristic of ALS tend to underestimate its presence. The BBI overcomes both limitations and should be considered the gold standard to assess behaviour in ALS.

## Hypothalamic atrophy correlates with onset of disease-defining symptoms in patients with ALS

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Background: Alterations in energy metabolism have been described in the context of ALS but direct evidence of hypothalamic involvement is lacking. The hypothalamus plays a key role in controlling the energy metabolism.
Objective: To study whether possible morphologic alterations of the hypothalamus in manifest ALS and asymptomatic ALS mutation carriers are associated with the course of the disease.
Methods: High-resolution whole-brain based T1-weighted 3D-MRI data together with clinical and laboratory parameters were acquired in a large monocentric cohort including 270 manifest ALS patients, 32 asymptomatic ALS mutation carriers, and 116 healthy controls. The hypothalamic volumes were semimanually quantified following a well-established procedure that was optimized to sensitively detect hypothalamic volume alterations. The hypothalamic volumes for all subjects were measured by an experienced rater who was blinded for any clinical information. The determined hypothalamic volumes were normalized for the intracranial volume and corrected for age.
Results: The hypothalamic volumes were significantly atrophied in both manifest ALS patients ($p<0.0001$) and asymptomatic ALS mutation carriers ($p<0.01$) compared with controls. There was no significant correlation with global brain atrophy, metabolic indices (among others BMI), or hypothalamic-associated hormones (leptin, adiponectin). In contrast, the disease onset was significantly correlated with the hypothalamic volume ($p=0.012$) and survival was better ($p=0.022$) the less the hypothalamus was atrophied.
Conclusions: The hypothalamus appears to be selectively atrophied even in an asymptomatic phase of ALS. The volume loss of the hypothalamus is likely to be associated with the onset of ALS-defining symptoms and possibly a prognostic factor for survival.

## A PET/CT approach to spinal cord metabolism

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Aim: Amyotrophic lateral sclerosis (ALS) is fatal late-onset neurodegenerative disorder of adult life, characterized by a progressive impairment of motor function. We recently developed new software able to recognize the spinal canal and spinal cord (SC) tracer uptake on PET/CT images. Our study aims to investigate whether this method permits to identify abnormalities in SC metabolism in ALS patients.
Materials and Methods: We studied 30 patients with spinal-onset ASL at different clinical stage, submitted to FDG-PET/CT following 1-36 months (median 14) after diagnosis. Obtained data were compared with corresponding findings in age and sex-matched healthy controls selected from a published normalcy database. Image analysis was performed according to a previously validated algorithm able to identify all vertebrae from the image data set and to extract the spinal canal as the non-osseous space within the spine volume. The output of the software was therefore the extraction and the 3D-representation of spinal canal volume that served as a mask to recognize SC using a segmentation algorithm based on Hough transformation. Thereafter, mean standardize uptake value (SUV) of cervical and dorsal SC, normalized to the liver (NSUV), was evaluated in comparison with normal subjects.

Results: No differences were found in SC volume between patients and controls. FDG uptake was slightly, yet significantly, higher in ALS patients in the whole SC (NSUV 0.82±0.28 vs 0.70±0.14 $p<0.05$) and in cervical segment (NSUV 0.99±0.37 vs 0.85±0.20, $p<0.05$). During follow-up 13 patients died. A potential prognostic role of SC metabolism was suggested by the observation of a higher SC_NSUV in non-survivors compared with the survivor patients (0.71±0.26 vs 0.55±0.16, $p<0.05$). Kaplan-Meyer approach confirmed the predictive value of SC-NSUV while multivariate analysis confirmed the additive nature of metabolic information (HR = 24.3, 95% CI 2.2-262.8). By contrast no association with prognosis was observed for age, ALSFRS-R score, time elapsed from diagnosis to PET scanning or presence/absence of riluzole treatment. Conclusion: Our computational approach might represent a new window to explore SC metabolism from PET/CT images. Whether confirmed in larger prospective studies, the prognostic significance of SC metabolic pattern in ALS patients might suggest a relevant role for SC inflammatory response in ALS progression.

## Role of FUS in post synaptic neuromuscular junction differentiation

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During ALS disease progression, the neuromuscular junction (NMJ), that is the specialized synapse between the motor neuron and the muscle fiber, is the first structure to be affected. Indeed, the NMJ is dismantled before degeneration of motor neuron cell body. Results from our laboratory also implicate muscle metabolic defects in the neurodegeneration observed in ALS patients and models. A subset of ALS cases is caused by dominantly inherited mutations in the gene encoding FUS, a RNA-binding protein involved in multiple steps of RNA metabolism. ALS-linked FUS mutations cause typical ALS, with young onset and rapid disease progression. Most of the known FUS mutations alter the import of FUS in the nucleus, and the mutations leading to the most severe clinical pictures are truncating mutations deleting the C-terminal nuclear localization signal (NLS). Our laboratory previously developed a conditional Fus Knock-In model of ALS (Scekic-Zahirovic et al., EMBO J, 2016; Scekic-Zahirovic, Acta Neuropathol, 2017). These mice display a constitutive deletion of NLS that can be rescued to the wild type situation upon CRE-mediated recombination. We observed that the complete cytoplasmic mislocalization of FUS in homozygous Knock-In mice leads to perinatal death, accompanied by motor neuron degeneration. Interestingly, rescuing FUS localization in motor neurons rescued motor neuron degeneration, yet perinatal death was not rescued. Our working hypothesis is that Fus mutation is primarily causing defects in NMJ structure and differentiation. Indeed in a cell model, FUS is both necessary and sufficient to drive expression of acetylcholine receptor genes of the NMJ and in an animal model, complete Fus mislocalization showed ultrastructural presynaptic defects at the NMJ. Besides presynaptic defects, muscles of these mice showed abnormal post-synaptic acetylcholine receptor clusters, and this was associated with defects in expression of a number of NMJ-related genes in muscles. Furthermore, adult heterozygous Fus knock-in mice, showing partial cytoplasmic mislocalization of FUS, display smaller endplates. So, proper FUS function is required for the normal neuromuscular junction differentiation. The understanding of the role of FUS in NMJ structure and function could be instrumental in designing therapeutic strategies for FUS-ALS.

## Developing vertebrate models to highlight the functional relevance of Nefl and miRNAs in ALS pathogenesis

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A striking number of genes, including TDP-43, FUS and ATXN2 implicated in ALS pathogenesis encode proteins with functions in RNA metabolism. Also, preliminary results suggest that miRNAs are aberrantly expressed in spinal motor neurons, and that mRNAs encoding neurofilament proteins are a disease relevant target and represent the most reliable prognostic biomarker for ALS patients. To address the mechanisms involved and identify common therapeutic targets, we developed zebrafish models, which allows large-scale drug screening and in vivo assessment of biological processes, combined with a wide range of genetic tools: gene overexpression (DNA or RNA injection), knock-down (antisense morpholino injection) or knock out (CRISPR/Cas9 and deletion mutants) to define in vivo the functions of miRNAs and neurofilament proteins, as well as the consequences of their disruption. We identified and characterized the zebrafish homologues for the low molecular weight neurofilament protein (Nefl) and assessed its expression within physiological and ALS pathological conditions. We also established that down regulation of a specific Nefl isoform in zebrafish using antisense oligonucleotides results in a strong ALS-like motor phenotype (motor axon atrophy combined to a paralysis reflected by deficits in the evoked swimming response). We are currently developing long-term Nefl KO using the CRISPR/Cas9 genome editing tools and obtained zebrafish lines from EZRC carrying mutations in the Nefl orthologues. We also assessed by Western Blot the expression of Nefl within ALS zebrafish models, and revealed that TDP43 knock-down leads to a strong decrease in Nefl protein. Our collaborators, Drs. Strong and Hornstein identified specific miRNAs that affect Nefl expression and aggregation in vitro and are known to be aberrantly expressed in ALS patients. In parallel, we are performing down regulations of these miRNAs of interest in zebrafish embryos to assess their biological consequences. Inhibition of two of these miRNAs led to motor deficits and aberrant branching of axonal projections from motor neurons in live zebrafish larvae, associated with a strong disruption of Nefl expression. These animal models will allow us to elucidate the role of these key actors in ALS pathogenesis and will provide relevant endpoints for future studies to identify novel therapeutics targets for ALS. Funding provided by the ERA-NET E-rare (ANR) project RNA-ALS and the ERC project ALS-Networks.

## Phase separation of C9orf72 dipeptide repeats perturbs stress granule dynamics

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Neurodegenerative diseases are characterized by the presence of protein inclusion bodies with a different protein content depending on the type of disease. Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are no exceptions to this common theme. In most ALS and FTLD cases the predominant species of aggregated proteins are RNA-binding proteins (RBPs) (1). Yet the exact processes underlying this pathological aggregation remain unknown. In previous work we have shown that nuclear transport factors are modifiers of arginine-rich DPR toxicity (2, 3). These data suggest that DPRs could perturb the nucleocytoplasmic transport system, eventually resulting in cytoplasmic RBP mislocalization. We wondered whether DPRs also could play a direct role in the aggregation of these DPRs. Recent studies have shown that RBP aggregation is likely initiated from a liquid-like phase separated (LLPS) state. We now show that arginine-rich DPRs can undergo such phase transition themselves in vitro (4). This process is length- and dose-dependent, and is mediated by counterions or polyaromates. Moreover PR and GR are capable of affecting the phase separation and aggregation behavior of disease-relevant RBPs in vitro. Lastly, we found that PR and GR induce stress granule assembly in cells, and affect their dynamics and protein content. Our findings suggest that besides perturbing RBP subcellular localization, arginine-rich DPRs could also directly affect the phase separation and aggregation of RBPs in C9 ALS/FTLD pathology. In recent work we are further exploring the physical underpinnings of arginine-mediated phase separations and further characterizing the role of RNA in this process. We have recently shown that RNA can be an active mediator of phase separation of disordered arginine-rich domains (4). Yet there remain a lot of unanswered questions, and the general view is that RNA is mostly a passive bystander in protein phase separation. We have preliminary data that RNA sequence and structure are however major determinants of LLPS, and are further investigating this in vitro and in cells.

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## Dynamic polymerization of TDP-43 in health and disease

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TDP-43 is a primarily nuclear RNA-binding protein (RBP), whose abnormal phosphorylation and cytoplasmic aggregation characterizes affected neurons in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Even though the loss of normal nuclear localization and cytoplasmic TDP-43 aggregation correlates with neurodegeneration, the exact mechanisms of neurotoxicity remain elusive. Moreover, the molecular mechanisms triggering TDP-43 pathology in ALS and FTD remain poorly understood, in part due to lack of high-resolution structural information of TDP-43 in the physiological as well as pathological state. Here we report that physiological TDP-43 exists as nuclear oligomers that are distinct from cytoplasmic complexes formed upon cellular stress or pathologic aggregates. To elucidate the molecular basis of physiological TDP-43 oligomerization, we determined the crystal structure of TDP-43 NTD at 2.1-Å resolution, which revealed an unprecedented mode of head-to-tail interactions between monomers generating solenoid-like polymers (1). Consistent with the crystal structure, solution NMR spectroscopy confirmed the dynamic nature of inter-molecular and low micromolar affinity electrostatic interactions that stabilize these polymers (1). Destabilizing oligomerization by point mutations resulted in loss of TDP-43 regulation of alternative splicing of known neuronal RNA targets, indicating that these dynamic TDP-43 oligomers are the functional form of the protein in vivo (1). Tripartite GFP complementation experiments in cells illustrate that physiological TDP-43 oligomerization prevents low complexity domain intermolecular interactions (1). Importantly, we show that NTD-driven TDP-43 oligomerization antagonizes pathologic aggregation. This dynamic head-to-tail polymerization of TDP-43 is unique among RBPs and broadens our understanding of TDP-43 function. Most excitingly, our findings indicate that stabilization of functional TDP-43 oligomers could have therapeutic potential by counteracting pathologic aggregation and restoring nuclear function.
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## Deciphering the function and mechanisms of C9ORF72 in ALS

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ALS has a major genetic contribution, the most common genetic abnormality being the GGGGCC hexanucleotide repeat expansion (HRE) in the C9ORF72 gene. Proposed mechanisms concerning C9ORF72 mutation pathogenicity are loss and gain of function including aggregation of dipeptide repeats (DPRs) translated through a non-ATG-initiated translation. One of the hypotheses would be a role in autophagy for C9ORF72. Interestingly, autophagy is also linked with other ALS causative genes such as VCP, UBQLN2, OPTN and TBK1. SQSTM1 is another gene mutated in ALS patients with a well-known cellular function. SQSTM1/p62, is an essential actor of the initiation of the autophagy pathway. p62+ inclusions have been detected in patients carrying the C9ORF72 HRE leading to the hypothesis of a functional common purpose between these genes. To investigate the pathogenic mechanisms induced by C9ORF72 and SQSTM1 mutations in ALS, we developed zebrafish models for these genes. Loss of function of the Sqstm1 and C9orf72 zebrafish orthologues leads to specific motor phenotypes associated with shorter motor neuron axons and reduced swimming capacity. To elucidate the common cellular mechanisms underlying autophagy dysregulation in motor neuron degeneration, C9orf72 and Sqstm1 zebrafish models were used to analyze their epistatic interactions. We found that C9orf72 and Sqstm1 partial inhibitions have an additive effect and that C9orf72 can rescue the phenotype obtained with Sqstm1 knockdown. These results indicate that both proteins belong to the same pathway and that C9orf72 is downstream of Sqstm1. Also, we found that C9orf72 depletion in mouse motor neurons primary cultures leads to the early death of motor neurons associated with their inability to product autophagosomes. To develop a vertebrate model of DPR pathogenicity, we expressed plasmids containing GFP-tagged expanded repeats for all DPRs: poly(GA), poly(GP), poly(GR), poly(PR), and poly(PA). We found out that the loss of function of C9orf72 is essential to trigger DPR accumulation in zebrafish. Inclusions of DPR happening under C9orf72 knockdown are associated with motor abnormalities - with fish losing their capability to swim, and with shortened motor neuron axons. These results indicate that DPR inclusions and C9orf72 knockdown act in a common pathogenic mechanism, suggesting that both gain and loss of function synergize in the C9ORF72 HRE pathogenicity and thus opening novel avenues for potential treatment of ALS.

## Live imaging of RNA dynamics for genetic forms of amyotrophic lateral sclerosis (ALS) in zebrafish

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The recent identification of mutations in TDP-43, FUS, two RNA-binding proteins, altogether with C9orf72 and SOD1 representing more than half of genetic cases, and also SQSTM1 and VCP, seems to unveil a global mechanism of alteration of both protein and RNA homeostasis leading to toxicity and cell death in ALS. The lab has specialized in studying and developping ALS models in zebrafish. The revelance of this model in the field of motor neuron diseases relies on being a practical, convenient and large progeny generating vertebrate model. Furthermore, this model shows a great conservation of genetic mechanisms with mammals and humans. Moreover, the zebrafish allows the use of a wide range of genetic tools convenient to develop transient and stable transgenic lines. Also, this model shows transparency at the embryonic stage, making it particularly relevant to study, especially through imagery, pathogenic mechanisms in vivo. This project aims to study key ALS transcripts such as Sqstm1, VCP or C9orf72 in order to potentially comprehend their implication in the pathology through the use of zebrafish disease models already established in the lab. Two in vivo techniques will be developped: -the MS2 system which relies on the interaction between bacteriophage's MCP protein (MS2 Coat Protein) and a specific "hairpin" RNA sequence called MBS (MS2-Binding site). The addition of several MBS sequences to an RNA sequence combined with the presence of the MCP protein fused to a fluorescent protein allows you to follow this particular exogenous RNA sequence at the cellular level (Buxbaum et al, 2015). -a modified Crispr system using an inactive Cas9 protein, fused with a fluorescent protein ("dead Cas9-eGFP"). Through modifications, the Cas9 is now adapted to target an endogenous mRNA thus making its visualization possible without modifying it (Nelles et al, 2016). The localization of transcripts through live-imaging will allow us the caracterization of key ALS RNA species metabolism and their defects in pathological mechanisms. All together with complementary experiments such as in situ colocalization with proteins, quantification of RNA levels, pull-down of interacting partners, novel aspects of disease pathogenesis leading to neuronal death would be unveiled. We believe a detailed study of RNA metabolism, especially through in vivo experiments, has the potential to durably impact our understanding of ALS and to a certain extent, other pathologies.

## Two G-quadruplex structures adopted by oligonucleotide model of ALS and FTD linked GGGGCC repeats

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The most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is a large increase in the number of GGGGCC repeats located within the non-coding region of C9orf72 gene. Non-canonical structures adopted by expanded GGGGCC repeats, including G-quadruplexes, have been proposed to be crucial in pathogenesis. G-rich oligonucleotides can form in the presence of K+ or Na+ ions non-canonical four-stranded structures called G-quadruplexes, composed of stacked layers of G-quartets that are formed by four guanine residues connected by Hoogsteen hydrogen bonds. G-quadruplexes are known to be structurally diverse and their folding sensitive to oligonucleotide sequence and experimental conditions. In addition, several G-quadruplex structures often coexist in solution, representing a great challenge for high-resolution structure determination. Oligonucleotide d[(G4C2)3G4], chosen as the shortest model with the ability to fold intra-molecularly, formed two major and several minor G-quadruplex structures. Structural polymorphism was reduced by dG to 8Br-dG substitution and led to stabilization of two structures in d[(G4C2)3GGBrGG]. Interestingly, relative populations of G-quadruplex structures were sensitive to pH and rate of cooling when folding from thermally denatured state in the presence of K+ ions. Two different folding conditions were established that selectively favor formation of mostly one of the structures, thus facilitating their individual structural characterization with NMR. Two G-quadruplex structures are topologically very similar, however, they exhibit unique structures and distinct dynamic properties.

## The role of valosin containing protein (VCP) in the clearance of toxic misfolded protein aggregates in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is classified in sporadic forms (sALS) present in 90 % of the cases and familial forms (fALS) present in the remaining 10% of the cases. Both forms are characterized by the presence of intracellular aggregates. sALS and fALS, except those related to Superoxide Dismutase 1 (SOD1), are characterized by inclusions positive for TAR-DNA binding protein 43 (TDP-43). These inclusions sequester essential cellular components and could impair the Protein Control Quality (PQC) system. The PQC system can also be altered by the mutation of genes that express proteins involved in the system. One of the genes found mutated in fALS is Valosin Containing Protein (VCP). VCP is a member of the AAA+ ATPase family that are chaperon-like proteins involved in the PQC system. VCP works as a homohexamer and contains two ATPase units and an N-terminal unit. The N-terminal unit interacts with a large number of adaptors that permits VCP to be involved in various pathways of the PQC system. VCP role is to route misfolded proteins mainly to the Ubiquitin Proteasome System (UPS) but recently VCP was found involved also in the autophagic pathway. Recent studies show that in VCP-ALS patients, as well as in other diseases where VCP is found mutated, the brain tissue is characterized by inclusions positive for TDP-43. Experimental work also correlated VCP-mutants to the impairment of the autophagic pathway, suggesting an involvement of VCP in the clearance of intracellular inclusion and its impairment in disease. Here we report that the overexpression of VCP in an ALS in vitro model enhances the clearance of mutated SOD1-aggregates mainly through the UPS. Moreover, VCP seems to have a role also in the removal of TDP-43 aggregates. In fact the chemical inhibition of VCP increases the aggregation of TDP-43. These data start to define VCP importance in the clearance of misfolded protein aggregates, which are the cause of cell-toxicity in ALS and other neurodegenerative diseases. These findings could make VCP a new potential target for ALS.

## Intranuclear (G4C2)n RNA foci, transcribed from C9ORF72 hexanucleotide expansion mutation, form paraspeckle-like structures

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Expansion of GGGGCC hexanucleotide repeat in the C9ORF72 gene is the most common pathogenic mutation in the families with autosomal dominant frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS) and FTD/ALS. The expanded repeat is transcribed from the sense and the antisense strand and forms intranuclear RNA foci. Here we show that the core paraspeckle proteins SFPQ, NONO and PSPC1 bind to (G4C2)n RNA in vitro, co-localise with intranuclear RNA foci in cells transfected with the expanded repeats and to a lesser extent in post-mortem brain tissue. Paraspeckles are nuclear structures functioning in nuclear retention of adenosine to inosine edited RNA. They form on a backbone of the long non-coding RNA NEAT1. We demonstrated that the presence of RNA foci increased the number of SFPQ stained subnuclear bodies, however only a small fraction of (G4C2)n RNA foci co-localised with NEAT1. Sense RNA foci lead to nuclear remodelling, as shown by an increased number of SFPQ-stained subnuclear bodies, which form independently of the known paraspeckle platform NEAT1. Thus, our results suggest that (G4C2)n RNA may lead to formation of paraspeckle-like structures, which may compete with NEAT1 for associated proteins and modulate nuclear compartmentalization of paraspeckle-bound RNAs.

## C9orf72 interacts with coilin and influences Cajal body dynamics and splicing

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Rationale & Hypothesis: The GGGGCC hexanucleotide repeat expansion in the C9orf72 gene is the leading cause of amyotrophic lateral sclerosis (ALS). Although the precise disease mechanisms are unclear, haploinsufficiency of C9orf72 has been proposed. Little is currently known about the function of the C9orf72 protein, but a yeast two-hybrid assay found an interaction between C9orf72 and coilin. Coilin is the major protein component of Cajal bodies (CBs), nuclear suborganelles which act as sites of splicing machinery maturation and assembly. C9orf72 ALS patients have splicing defects that correlate with disease severity. We hypothesise that haploinsufficiency of the C9orf72 protein in ALS leads to dysregulation of Cajal bodies and downstream splicing.
Objectives: Confirm the interaction between C9orf72 and coilin and investigate the effect of C9orf72 on Cajal bodies and splicing.
Methodology: An in vitro GST-binding assay was used to establish if there is a direct interaction between GST-C9orf72 and 35S-labelled coilin. Co-immunoprecipitation experiments were performed on Hek293 cell lysates to confirm the cellular interaction between myc-C9orf72 and endogenous coilin. The localisation of the interaction was investigated using an in situ Proximity Ligation Assay (PLA). Immunostaining for coilin and SMN was performed on Hek293 cells following C9orf72 knockdown to investigate the effect of C9orf72 on CBs. Finally, an artificial reporter splicing assay was used to study the effect of C9orf72 knockdown on splicing efficiency.
Findings: The GST-binding assay suggests there is a direct interaction between C9orf72 and coilin in vitro. In cell lysates, myc-C9orf72 was successfully co-immunoprecipitated with endogenous coilin, suggesting the interaction is physiologically relevant. PLA confirmed the interaction and shows it occurs in both the cytoplasm and nucleus. Interestingly, C9orf72 knockdown in Hek293 cells led to an increase in the number of CBs but a decrease in the splicing of both major and minor introns in the artificial reporter splicing assay. The results confirm C9orf72 interacts with coilin and may regulate CBs and splicing.

## Direct RNA toxicity in a transient zebrafish model of C9orf72 ALS is abrogated by PURA and p62

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A repeat expansion in the C9orf72 gene is the most frequent cause of ALS. This repeat expansion is believed to be neurotoxic because of (a combination of) direct RNA toxicity, DPR toxicity or loss of function. We aimed to investigate the role and mechanism of direct RNA toxicity in an in vivo model. Injection of both sense and antisense repeat RNA constructs into zebrafish oocytes induced a motor neuronal phenotype at 30 hours post fertilization. Using dot blot, western blot and ELISA, generation of significant amounts of DPRs was excluded. Although forced expression of DPRs (in particular GR and PR) could induce a motor neuron phenotype, no GR and PR were detected in fish injected with repeat RNA. Moreover, synergic toxicity between low (not toxic) levels of the different DPRs was excluded. Additionally, interrupted repeat RNA constructs which are unable to generate DPRs were found to be neurotoxic as well. Hence, this transient zebrafish model seems to be a model for C9orf72 related direct RNA toxicity. To investigate the mechanism of this direct RNA toxicity we performed an RNA pull down to identify repeat RNA binding proteins. PURA was confirmed as binding partner of GGGGCC repeats. Direct RNA toxicity, but not DPR toxicity, was prevented by overexpression of PURA. This beneficial effect depended on its glycine-rich and PUR2 domains. PURA was found to increase the levels of the autophagy related protein p62 in both C9orf72 and control fibroblasts, and this effect was dependent on the PUR2 domain. Moreover, direct RNA toxicity in zebrafish was prevented by p62 overexpression. Interestingly, sense repeat RNA was found to induce stress granule formation in HEK cells, and PURA was found to prevent stress granule formation. This effect was dependent on the glycine-rich domain of PURA. In conclusion, we provide evidence for direct RNA toxicity of the C9orf72 repeat expansion using a transient zebrafish model. We show that the RNA binding protein PURA prevents this RNA toxicity and that this protective effect is likely mediated through modulation of autophagy and stress granule dynamics.

## Secretion of toxic exosomes by muscle cells of ALS patients: role in ALS pathogenesis

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Background: The causes of ALS remain unknown. For sporadic and familial cases, several studies show an abnormal accumulation of lysosomally-directed protein aggregates in the cytosol. Mutations in genes involved in the autophagy pathways and multivesicular body biogenesis are now known to occur among familial cases. These data suggest a potential disruption of the endosome and lysosome pathways, thus affecting exosome genesis. Work in our lab and others has shown that the skeletal muscle has a functional secretory activity. The muscle secretome contains exosomes - vesicles that carry out intercellular transport of functional proteins, mRNA, and miRNA.
Aims: We hypothesize that exosome secretion is disrupted in ALS muscle cells, and that this affects the intercellular communication between muscles and motor neurons.
Materials & Methods: Muscle stem cells were purified from ALS, SBMA, SMAIII-IV and age-matched healthy subjects (n=18, n=12, n=12, n=21 respectively). Exosomal toxicity was tested by adding them to the culture medium of healthy human muscle cells, human IPS-derived motor neurons or primary murine motor neurons.
Results: Whereas clinically overlapping pathologies SBMA and SMA-IV have gene expression profiles similar to healthy controls, myotubes of sporadic ALS patients have a strongly specific gene expression signature. The 30 genes most strongly contributing to this ALS-specific signature encode proteins localized to a form of secreted vesicle known as the exosome. We confirmed by RT-qPCR, immunostaining and electron microscopy that the exosomal content is significantly upregulated in both ALS

myotubes and muscle biospises. In electron microscopy of sporadic ALS muscles cells we observed multi-vesicular bodies that are significantly more filled with exosomes (1.40 ± 0.14 exosomes/mm2 in ALS, 0.9 ± 0.07 exosomes /mm2 in control), and ALS myotubes released 2-fold more exosomes than healthy controls. ALS exosomes added to the culture medium of healthy muscle cells or of healthy motor neurons induced: (1) muscle fiber atrophy, (2) cellular stress by stimulating membrane blebbing, (3) cell death of muscle cells and motor neurons.
Conclusion: The secretion of toxic exosomes occurs independently of muscle denervation and could be a potential mechanism to explain the progressive spread of the disease across muscles and motor groups.

**PI**

## TDP-43-based biomarker development in ALS

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Background: Identification of biomarkers is needed to facilitate effective therapy development in ALS. TDP-43 is a normal protein that abnormally accumulates in nerve cells of nearly all cases of ALS and half of those with the linked condition of frontotemporal dementia (FTD). Measuring the disease-relevant form of TDP-43 in the blood and spinal fluid of ALS patients has not been successful so far. Identification of the pathological forms of TDP-43 across ALS-relevant biofluids and tissue is an important step in improving the potential of this approach.
Method: Immunoprecipitation of TDP-43 with a polyclonal full-length TDP-43 antibody from paired CSF and serum samples from a group of ALS patients and healthy controls within the Oxford Study for Biomarkers in MND ('BioMOx'). Immunoprecipitation of TDP-43 from brain homogenate taken from a (BAC)-transgenic mouse model carrying GFP-tagged human TDP-43 (M337V mutant and wildtype TDP-43).
Results: By immunoblotting native mouse brain homogenate, a 48 kDa band and a higher 70kDa was observed using a full-length TDP-43 antibody. Immunoprecipitation using the anti-TDP-43 antibody showed an immunoreactive band at 48kDa and a stronger band at 70kDa. No immunoreactive bands at 48kDa and 70kDa were observed in the negative immunoprecipitation control using IgG rabbit isotype control as bait antibody. After immunoprecipitation of TDP-43 from serum, the immunoblot showed a 43kDa TDP-43 immunoreactive band. This band was not detected in native serum, supernatant and the negative immunoprecipitation control applying IgG rabbit isotype control as bait antibody. The 25 and 55-60kDa bands represented co-eluted antibody light and heavy chain peptides.
Conclusion: The systematic enrichment of TDP-43 from ALS patient biofluids and tissue of ALS-relevant disease models is necessary to identify the disease-relevant TDP-43 forms and to enable downstream analysis of TDP-43 peptides towards the goal of biomarker development.

P3

## Phosphorylated neurofilament heavy chains in blood as biomarker for ALS?

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Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of upper and lower motor neurons. The phosphorylated neurofilament heavy chains (pNfH) are a major component of the cytoskeleton in the axon of (motor) neurons. Recently, increased pNfH levels in CSF have been proposed as diagnostic measure for ALS. Peripheral blood is a very accessible source that allows pNfH to be exploited as biomarker for ALS earlier in the diagnosis of ALS. We explored pNfH in blood as a candidate biomarker for ALS. Paired serum and CSF pNfH levels of ALS patients (n=79) and neurological disease controls (NDC) (n=72) were measured with an ELISA (Euroimmun, Lübeck, Germany). pNfH levels in CSF and serum correlated significantly ($r=0.61$; $p<0.0001$; $n=150$). Secondly, pNfH levels in serum were significantly higher in ALS patients compared to NDC (median (IQR), ALS: 174 pg/mL (70 – 351 pg/mL); NDC: 33 pg/mL (11 – 66 pg/mL); p(Mann-Whitney U test)<0.0001). Receiver operator characteristic (ROC) analysis determined an optimal cutoff of 85 pg/mL to discriminate ALS patients from NDC, with a sensitivity of 73% (CI: 62 – 83%) and a specificity of 81% (CI: 70 – 89%). The corresponding area under the ROC curve was 0.81 (CI: 0.74 – 0.88). These findings show that pNfH can be quantified in a reliable way in blood of ALS patients and NDC. Our findings warrant further research in blood-based biomarkers for ALS to discriminate ALS from genuine ALS disease mimics in the early diagnostic phase. Blood-based biomarkers will give insight on whether biomarkers can significantly decrease the diagnostic delay of ALS, promise to have applications as prognostic tool and as blood-based biomarker in drug development.

**P5**

## Proteomic analysis in postmortem neurological tissue of subjects with amyotrophic lateral sclerosis (ALS)

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Objectives: In this study we carried out a proteomic analysis in samples of postmortem brain tissue from donors with a clinical and pathological diagnosis of ALS, compared to 2 control groups: deceased donors without a neurodegenerative disease and donors with ubiquitin-positive frontotemporal lobar dementia (FTLD-U). The main objective of the study was to identify potential diagnostic biomarkers of ALS by analyzing protein expression in areas of neurological tissue with motor neuron degeneration. Furthermore, we intend to contribute to a better understanding of the pathophysiology of ALS and to assess the existence of differential mechanisms in the protein expression of subjects with ALS with respect to subjects with FTLD-U, as both are neurodegenerative diseases which share a common pathogenic mechanism.
Materials and methods: Eight subjects from each patient group were selected randomly. Neurological postmortem tissue extracted from the Neurological Tissue Bank of Navarre was used. We obtained frozen tissue of the anterior horn of the spine, the medulla oblongata, the motor and the non-motor frontal cortex. The Label-Free differential proteomic analysis was used to carry out the differential proteomic analysis. Immunohistochemistry and Western-Blot were used as alternative validation techniques. The IngenuityPathwayAnalysis software was used for the functional interpretation.
Results: A total of 2318 proteins were identified in the anterior horn of the spine, of which 1002 were quantified. We found 248 differentially-expressed proteins among cases of ALS versus healthy controls, and 33 among cases of FTLD-U versus healthy controls. Of these differential proteins, 20 were shared between both groups (ALS and FTLD-U). For the functional interpretation of the results, those proteins seen to be differentially expressed in cases of ALS were analyzed with Ingenuity in order to determine the networks of protein interaction, metabolic pathways and their implication in other pathological processes. The preliminary results proved the involvement of these proteins in cell survival, mitochondrial respiration and specific neuronal functions.

Conclusion: We identified new proteins that seem to be involved in the pathophysiology of ALS. These results open new lines of research in the search for a potential diagnostic and prognostic biomarker of the disease, as well as for the identification of the underlying molecular mechanisms involved in the pathogenesis of ALS.

**P7**

## Internal control genes validation for qPCR analysis in lymphocytes from patients with amyotrophic lateral sclerosis

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Quantitative polymerase chain reaction (qPCR) is the most sensitive and reliable method for determination of mRNA gene expression. However, alternations in the amount of starting material, RNA extraction, efficiency of reverse transcription and amplification may result in quantification errors. For this reason the choice of adequate internal control genes (ICGs) is the most important part of correct normalization. There are no universal ICGs with stable expression among different tissues and various conditions. In many cases housekeeping genes are not good ICGs, since they may undergo significant regulation during cell differentiation or pathological processes and may vary between patients. Using random, popular ICGs without a proper validation of their expression stability leads to incorrect results. Despite the great efforts, the molecular mechanisms of sporadic ALS remain unclear and a specific disease marker is still lacking. Lymphocytes are an easily accessible source of material both for genetic and molecular studies. Moreover, establishment of lymphoblast cell lines (LCLs) by EBV transfection potentially provides an unlimited source of biologic material. The aim of the study was to evaluate the usefulness of eight commonly used ICGs (18SRNA, ACTB, B2M, GUSB,GAPDH, HPRT1, MT-ATP6 and RPS17) in gene expression analysis in ALS. The study was performed in 26 ALS patients and 30 control subjects. The peripheral blood mononuclear cells (PBMCs) before and after immortalization (LCLs), and LCLs before and after liquid nitrogen cryopreservation (LCL1 and LCL2, respectively) were used for qPCR. The qPCR raw data was analyzed by statistically-based algorithms: BestKeeper, NormFinder and geNorm. All studied genes were expressed in both ALS and control PBMCs, whereas only four (18SRNA, GAPDH, MT-ATP6 and RPS17) in LCLs. Comprehensive ranking of three methods indicated RPS17 and MT-ATP6 as the best ICGs pair for qPCR in PBMCs of both control and ALS subjects, and RPS17 with 18RNA or MT-ATP6 in LCLs from patients with ALS. In contrast, the most variable genes in PBMCs were ACTB and 18SRNA. We recommend using RPS17 and MT-ATP6 in qPCR studies performed simultaneously on PBMCs and LCLs.

P9

## Blood polyunsaturated fatty acid composition is a biomarker for amyotrophic lateral sclerosis

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Objective: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease associated with important disarrangements of energy homeostasis and lipid metabolism. Here we determined the polyunsaturated fatty acid (PUFA) composition of lipids in blood samples to establish metabolic biomarkers of the disease.
Methods: We recruited 134 patients with ALS, 48 age and gender matched healthy volunteers, and 12 patients with spinal and bulbar muscular atrophy (SBMA). We analysed PUFAs extracted from total lipids in serum and clotted blood cells by gas chromatography. We performed analysis of ROC curves to assess diagnostic potential, and applied univariate and multivariate models to estimate effects on survival.
Results: We observed a significant decrease in PUFA composition of blood cell lipids in ALS patients relative to control subjects, particularly affecting levels of docosapentenoic acid (DPA, 22:5n-3) and arachidonic acid (AA, 20:4n-6). Relative indices of ω3 and ω6 PUFA biosynthesis (EPA/ALA and AA/LA, respectively) also diminished significantly. Similar differences were found when ALS patients were compared to patients with SBMA, which is an ALS mimic condition. These observed modifications satisfied high standards for diagnostic purposes (95.8% specificity, 66.7% sensibility, LR(+)=15.9 and LR(-)=0.35 for DPA in ALS versus healthy subjects; and 92.9% specificity, 75.0% sensibility, LR(+)=10.6 and LR(-)=0.27 for AA/LA ratio in ALS versus SBMA patients).

Moreover, variations in the serum of ALS women, but not men, of levels of total ω3 PUFA, ω3/ω6 ratio and ω3 index (DHA, 22:6n-3 + EPA, 20:5n-3) were independent prognostic factors for survival. Patients with low rates of these parameters had a prolonged survival of more than 10 months compared to patients with high rates. Conclusion: Detailed analysis of PUFA composition of blood lipids revealed important metabolic biomarkers for ALS that could used both in clinical practice and in clinical trials.

**PII**

## The synergistic effect of IL-2, IL-6, IL-10, IL-13 and eotaxin influence longevity in transgenic SOD1G93A mice

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The role of inflammatory biomarkers in the progression of amyotrophic lateral sclerosis (ALS) is growing evidence during the last decade. Despite origin of ALS remains unknown, multiple panels of inflammatory biomarkers have been described in ALS patients and murine models to explain the progressive motor neuron loss and muscle atrophy. The purpose of this study was to identify biomarkers of inflammation that can influence disease progression and longevity in serial blood samples taken from transgenic SOD1G93A mice. Serial plasma samples were obtained from twenty SOD1G93A male and female mice at early symptomatic (60 days old) (P60), symptomatic (90 days old) (P90) and terminal or endpoint stages (from 112 to 144 days old). Plasma samples were analysed by a multiplex immunoassay to study protein expression of interleukin (IL)-2, IL-6, IL-10, IL-13, eotaxin, receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage inflammatory protein (MIP)1-a. Immunoassay results revealed a continuous increase of eotaxin levels throughout disease progression in transgenic SOD1G93A mice. Additionally, high levels of IL-2, IL-6 and eotaxin significantly increased the mortality risk in transgenic SOD1G93A male mice, while IL-10 and IL-13 levels exerted a dual role depending on the disease stage in transgenic males. Our findings showed for the first time in this ALS animal model that increased levels of IL-2, IL-6 and eotaxin were associated with a shorter life span of transgenic SOD1G93A mice. Furthermore, high levels of IL-2, IL-6, IL-10, IL-13 and eotaxin modulated the survival time in transgenic male mice, supporting the role of immune system in the disease progression. These novel findings could be relevant for a better understanding of the disease and for the development of accurate therapeutic strategies targeting biomarkers of inflammation.

**PI3**

## Expression of macrophage scavenger receptor (MSR I) in patients with motor neuron disease

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Introduction: The macrophage scavenger receptors (MSR1) bind a large variety of ligands including oxidized low-density lipoproteins (ox-LDL) and apoptotic cells. They control macrophages to prevent inappropriate activation of host cells and excessive tissue damage. MSR1 are involved in lipid metabolism and distribution. Hypercholesterolemia and high level of LDL has been observed in patients with motor neuron disease (MND).
Aims: The aim of the study was to analyze the expression of MSR1 gene in patients with normal and increased level of lipid parameters and various clinical phenotypes of MND.
Material and methods: The study included 72 patients with MND (26 females and 46 males, mean age 54.13 years) and 55 control subjects (27 females and 28 males, mean age 60.5 years). Diagnosis delay, disease duration, site of onset (bulbar/limb), phenotype (classic; progressive muscular atrophy, PMA; progressive bulbar palsy, PBP), functional status (ALS-FRS) and El Escorial criteria were analyzed. Laboratory parameters included total cholesterol (TCH), high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides (TG). The expression of MSR 1 was determined in peripheral blood mononuclear cells (PBMCs) using qPCR.
Results: Hypercholesterolemia was found in 64% and increased level of LDL in 44% of all MND patients. MSR1 expression was significantly higher in patients with normal level of TCH, as well as LDL when compared to patients with high level of each parameter ($p<0.05$). The MSR 1 expression did not depend on age, gender, site of onset, disease duration or TG concentration. The highest expression of MSR1 was found in classic ALS and the lowest in PMA. MSR1 expression was significantly higher in patients with definite MND, with ALS-FRS <30 and the diagnosis delay <12 month.
Conclusion: Since the denervated muscles obtain energy from circulating lipoproteins, the increased MSR1 expression in patients with advanced MND stage and severe disease progression may be due to the increased tissue energy requirement.

**PI5**

## Genetic and constitutional factors are major contributors to substantia nigra hyperechogenicity in ALS and other neurodegenerative diseases

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Hyperechogenicity of substantia nigra (SNh) is a frequent finding in amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and other movement disorders (MD) patients, but its meaning is unclear. To ascertain the contribution of different factors to SNh area, we measured it in 108 ALS, 102 PD, 91 other MD patients and 91 healthy controls. Demographical data were collected in all patients and controls. In ALS patients, we also recorded clinical variables, performed genetic analysis and measured baseline levels of ferritin. After family history and genetic testing, ALS patients were classified as familial (15) or sporadic (93). ALS, PD and other MD patients had a larger SNh area than controls. Left SNh and male gender, but not age, associated with larger SNh area in both patients and controls. Familial ALS patients showed larger SNh area than sporadic ones and familial ALS was the only clinical variable in the multivariate analysis to be associated with larger SNh area in ALS patients. Our results suggest that SNh associates with genetic and constitutional factors (male gender, handedness), some of which predispose to certain neurodegenerative diseases. This evidence supports the idea of SNh as an inborn marker of unspecific neuronal vulnerability.

PI7

## The TGFß- system – a critical factor in disease progression of amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) represents an orphan disease with high unmet medical need. Despite increasing knowledge on pathophysiology including genetic and molecular changes, epigenetics, and immune dysfunction, inflammatory processes may contribute to the heterogeneity and dynamics of ALS. Like other neurodegenerative disorders, ALS exhibits increased circulating TGFß- levels within the cerebrospinal fluid. Since the TGFß- system is involved in different essential developmental, physiological, immunological and fibrotic processes, it has emerged as a potential therapeutic target for ALS. We could demonstrate that an enhanced TGFß- system activity critically mediates the imbalance of neuroregenerative and neurodegenerative processes in ALS. The analysis of patient sera and post mortem tissue samples demonstrated an enhanced neurodestructive immune profile in ALS patients compared to healthy controls, as reflected by an increased expression of pro-inflammatory versus unchanged or reduced levels of anti-inflammatory cytokines. Further, sera of ALS patients displayed enhanced levels of angiogenic and vascular factors, indicating an enhanced pro-inflammatory and profibrotic activity. We investigated the activation state of the TGFß system in human sera and post mortem spinal cord, motor cortex, and occipital lobe, homogenates from ALS patients and controls (MND-net, MHH). Therefore, the expression patterns of TGFßRI, II, and the ligands TGFß1, TGFß2 were determined via qRT-PCR, Western Blot analysis, and electrochemiluminescence. Next, we examined the state of the immune system with respect to a neurotoxic or neuroprotective profile via electrochemiluminescence. Further, neuronal stem cell and neurogenesis markers were investigated in tissue homogenates via qRT-PCR and Western Blot analysis. In addition, to investigate fibrosis as a potential further hallmark of disease progression in ALS, we examined the expression profile of two major components of the extracellular matrix, Fibronectin and Collagen IV. The differences concerning ECM and actin-cytoskeleton of ALS patients and controls within the three different tissues were analyzed by qRT-PCR, Western Blot analysis, and immunofluorescence. Taken together our data

demonstrate that TGFß indeed may represent a critical factor for disease progression in ALS. Therefore, we conclude, the TGFß-system represents a very promising target for the treatment of ALS.

**PI9**

## Use of noninvasive ventilation in the treatment of ALS in Europe versus in the US: results of an international ALS specialist survey

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Amyotrophic lateral sclerosis (ALS) patients develop respiratory insufficiency as their disease progresses, and noninvasive ventilation (NIV) improves quality of life and extends survival. However, differences in clinical practice may impact timing for prescribing NIV. The objective of this survey was to understand preferred clinical practices including the criteria used to establish need for NIV and potential obstacles with its initiation and use in ALS. A 25-item questionnaire was sent via SurveyMonkey® to ALS specialists identified through membership in ENCALS (Europe) and NEALS (US); 48 responses from 12 European countries and 71 responses from the US were received. Descriptive statistics/comparisons between European/US responders were summarized. When considering NIV, both European and US specialists use upright FVC most but second-most important assessments differed. In Europe it was overnight pulse oximetry (6th in US) and, in the US, it was maximum inspiratory pressure (Europe 5th). Without insurance/financial constraints, fewer European than US specialists (Europe: 6/39 [15.4%]; US: 44/57 [77.2%]; $p<0.001$) would alter when they prescribe NIV. In patients with no respiratory symptoms, there was greater variability among European than US responses regarding the upright VC value at which NIV would be initiated. European responses were diverse: 10/39 [25.6%] would initiate when VC was <50% predicted, 9/39 [23.1%] at <60% predicted, and 8/39 (20.5%) would not initiate without respiratory symptoms, while most US specialists initiate NIV at an upright FVC/SVC <50% predicted (US: 41/60 [68.3%]). The most important reason for European specialists for recommending NIV was symptoms of orthopnea or

dyspnea, while it was upright FVC for US specialists. European specialists more often admit patients to a hospital for NIV initiation (Europe: 16/39 [41.0%]; US: 0/57 [0%] p<.001); US specialists more often refer patients to agencies that provide home trials/instructions (US: 39/57 [68.4%]; Europe: 5/39 [12.8%]; p<.001). NIV prescribing differs between Europe and the US and may be influenced by insurance/financial constraints. It is not known if these differences impact adherence with NIV usage and its effectiveness. As optimal NIV use may influence patient survival, the differences we found in Europe and the US can potentially confound results in ALS treatment studies.

P21

## Living wills for amyotrophic lateral sclerosis's disease patients: a psychological and integral health care perspective

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Background: Patients suffering from Amyotrophic Lateral Sclerosis (ALS) eventually lose their ability to communicate their treatment preferences in advanced stages of the disease. A living will empowers ALS patients to specify their choices concerning life-sustaining treatment beforehand. From the Department of Psychology of the Miquel Valls Foundation we provide emotional support to patients and their families in making decisions, helping to manage the different emotional states in order to maximize personal resources and to enhance the perception of control and resolution of obstacles arising from the disease.
Objectives: To describe and assess treatment preferences, sociodemographic and clinical variables and emotional state pre and post of living wills from ALS patients.
Methods: Descriptive and comparative cross-sectional study.
Results: There seems to be a homogeneous distribution of gender that decides to specify their living wills: there were 10□ and 10□. 80% of them were married and average age was 61.4 years old. Most part of the patients had invasive measurements and bulbar palsy when they were writing living wills. More than 50% rejected artificial feeding and 90% rejected invasive ventilation. None of them were in favour of body donation and 50% supported nervous tissue. Nearly 50% would rather die at home and almost 90% rejected spiritual assistance. 85% of patients were in favour of euthanasia just in case it would be legal. The average of time from the living will's writing to death was 2.34 years. It seems that writing a living will calm patients down and gives them control feelings.
Discussions and conclusions: It is known that both the patient and the family can face the last moments of the disease with anxiety. Living wills are a method to fulfil individual's wishes regarding end-of-life health. Our results denote the necessity and importance of an open and truthful patient-specialist communication which is a prerequisite for the discussion of living wills.
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P23

## Secular trends of ALS incidence in an Italian population-based register, 1995-2014: evidence for a birth cohort effect in women

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Aims: To evaluate the 20-year ALS epidemiological and demographic trends in the Piemonte and Valle d'Aosta Register for ALS (PARALS), a prospective ALS epidemiological register in Italy.
Background: Epidemiology of ALS has been widely studied in western countries but data on long term epidemiological trends are lacking.
Methods: ALS cases meeting El Escorial diagnostic criteria were enrolled using multiple sources in the period 1995-2014. Incidence rates were standardized to the 2001 Italian census population. The age-period-cohort (APC) effect was assessed with a partial least squares regression method and confirmed using a multi-phase analysis (median polish and a linear regression of the residuals to quantify the relative weight of the cohort effect).
Results: A total of 2702 ALS patients were diagnosed in the study period (1456 men, 1246 women). The mean age at onset was 65.7 (SD 11.1), with a 1.2 years increase from 1995-2004 (65.1, SD 11.1) to 2005-2014 (66.3, SD 11.1) (p=0.002). The mean annual crude incidence rate was 3.03 (2.85- 3.22)/100,000 population, increasing from 2.83 (2.66-3.01) (1995-1999) to 3.21 (3.02-3.42) (2010-2014). Also the standardized incidence rate increased from 2.67 (2.51-2.84) (1995-2004) to 2.85 (2.68-3.03) (2010-2014). The observed trend was mostly due to the raise of the incidence rate in women (2.37 [2.17-2.58] vs 2.63 [2.44-2.84]) while the incidence rate remained substantially steady among men (3.01 [2.79-3.31] vs. 3.06 [2.85-3.29]). Both APC models showed an increase of risk of ALS in women born between 1925 and 1934, while no differential risk was found in men.
Discussion: The increase of ALS incidence in the 20-year period of the study may be mostly ascribed to the increase of the median age of the referral population. However, we identified an increased risk in the 1925-1934 age classes in women, supporting the notion that environmental factors play a role in ALS pathogenesis.

**P25**

## Dry mass slope is a predictive factor in amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, associated to a metabolic impairment. Bioelectrical Impedance Analysis (BIA) of ALS patients showed that body fat, lean mass, or angle phase are potential biomarker of the disease. These variables are linked to some biochemical variables of interest in ALS (plasma cholesterol or creatinine).
Objective: To determine the variation of BIA parameters, and their link to the biological and clinical variables in ALS.
Materials and methods: Biological, BIA, and clinical data of ALS patients were collected every 3 months over the 18 months following the diagnosis. Repeated measures ANOVA was performed to determine within-patient variation of BIA variables over time. A cox model was used to perform univariate and multivariate analysis of patients survival.
Results: At baseline, a positive correlation between forced vital capacity and lean mass was observed ($p=0.0002$). Lean mass also correlated with plasma creatinine levels ($p=0.0022$). During the follow-up, a decrease of total metabolism ($p<0.0001$) and dry mass was observed ($p=0.0015$). The variation of dry mass was associated with survival, even after correction for BMI variation and diagnostic delay (HR=0.81 per kg of annual decrease; $p=0.0040$).
Discussion: This work shows for the first time the high importance of dry mass in ALS patients follow-up. It is also the first study to shed light on the relationships between biochemical and bioimpedance biomarkers in this disease. Finally it provides novel data on the longitudinal evolution of bioimpedance analysis variables over ALS evolution.
Conclusion: In this study, dry mass annual variation was an independent factor of survival. Bioimpedance analysis may thus be a valuable tool for follow-up of ALS patients, appropriately completing the biological follow-up.

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## The natural history of dysphagia in ALS: a population-based study

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Aim: To define the natural history of dysphagia in a population-based series of Italian ALS patients.
Methods: A total of 1236 patients with ALS from the Piemonte/Valle d'Aosta Register for ALS, incident in the 2007-2015 period, were considered. Cases were classified according to established site of onset: bulbar, upper limb, lower limb, respiratory. Disease progression was measured by the ALS-MITOS system, directly deduced from the ALSFRS-r score assessment three-monthly based. The time to gastrostomy was collected using multiple sources (hospital discharge records, outpatients visits/calls,..) and it was recorded in the register.
Results: The time to reach severe swallowing involvement (MITOS-EAT=1) was significant lower in female patients (median time 2.06 years in F vs 2.53 years in M; $p< 0.001$) and it progressively increased with aging (chi-squared 54,01, $p< 0.001$).
It was significantly lower in bulbar onset (median time 1.24 years) and respiratory onset (median time 1.36 years) patients compared to patients with upper/lower limb onset (median time 3.18 years, 4.34 years respectively). Also cognitive impairment was related to an early swallowing dysfunction, particularly ALS-Bi (median time 1.88 years, SD 0.63) and FTD (median time 1.58 years, SD 0.12). c9orf72 patients showed a significantly swallowing impairment compared to patients carrying other genetic mutations and wild-type patients (c9 median time 1.87 years, SD 0.25 vs wild-type median time 2.25 years, SD 0.11). 417 patients (33.3%) underwent gastrostomy in a median time of 1.86 years (SD 0.6) from onset. The gastrostomy time was significantly lower in respiratory onset patients (median time 0.79 years, SD 0.15) and bulbar patients (median time 1.67 years, SD 0.06).
Discussion: Bulbar dysfunction in ALS patients seems to be influenced by different demographic, genetic and cognitive aspects, underlining the complexity of pathological mechanisms involved in the phenotype determination. Moreover time to gastrostomy seems to be influenced not only by bulbar impairment (median time to PEG 1.86 years vs MITOS-EAT =1 time 2.25 years), but also by other clinical features (i.e. respiratory failure, weight loss).

P29

## Spatio-temporal assessment of the association between environmental exposures and the occurrence of amyotrophic lateral sclerosis (ALS)

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Background: Although the causes of ALS are unknown, an interaction between genetic and environmental factors may be involved in its development. Systematic reviews confirm a heterogeneous distribution worldwide of ALS. In fact, there is systematic evidence of the existence of spatial clusters of ALS, some of them associated with some environmental factor (heavy metals, electromagnetic radiation, pesticides, etc.). However, with the exception of pesticides, the systematic evidence of the association between environmental factors and ALS is inconsistent. Our objective here is twofold, to provide evidence of the existence of spatial clusters of ALS and on the association between environmental factors and these clusters.
Methods: In our study, we use a case control study (284 cases, 47.3% women). The cases were subjects with an ALS diagnosis between 2011 and 2016. Cases were matched with controls by sex and year of birth. As explanatory variables of interest we included environmental variables: distance (from the subject's home address) to roads (stratified into three categories according to the traffic intensity), distance to gas stations, distance to agricultural areas, distance to green zones, and environment noise levels (daytime, evening and nigh time). We control for both observed (sex, age, body mass index, weight loss and a contextual deprivation index) and unobserved confounders. We allowed the relations between the variables of interest and the deprivation index to be non-linear, modelling them both parametrically and non-parametrically. We controlled unobserved confounding by introducing random effects, which capture individual heterogeneity (at the individual level and at family level), temporal dependence (date of onset of symptoms) and spatial dependence (through a Matérn covariance structure. Inferences were performed using a Bayesian framework through the INLA approach.
Results: As preliminary results, we observed certain spatio-temporal clusters of ALS. The risk for subjects living near major traffic roads was around 10% higher than those who lived further away. The excess risk ceased to be statistically significant at around 1300 meters of major roads.
Conclusion: Our preliminary results indicate that the spatio-temporal clusters of ALS could be associated with some environmental factors, such as living near major roads.

**P31**

## Advance directives and the decision-making process in an ALS Unit in Spain

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to death between 3-5 years from symptoms onset. Treatment is mainly supportive, with some invasive procedures that can prolong survival and improve quality of life. The deliberative decision-making model requires the involvement of health-care professionals (HCP) in a continuous iterative process of bidirectional information that helps to make the patient's own decision about these procedures. We aimed to analyse patients and HCP beliefs, values and their preconceptions about invasive procedures, in order to improve the decision-making process. First, we designed a specific document of advance directives for ALS patients. This document explores the two main decisions about invasive procedures in the disease process (PEG and tracheostomy) as well as other important aspects about end-of-life management. From 2016, this document is provided to ALS patients treated in La Fe Hospital as a part of our decision-making care plan and their wills are recorded in a database. Then, HCP involved in the decision-making of ALS patients in the Valencian Region were also contacted with an online survey to explore their beliefs and views about invasive procedures in ALS. Thirty patients filled and signed our advance directives document and fifteen health care professionals completed the survey. Patients were younger, more frequently males and believers than HCP. A great majority of patients and HCP declared they would accept the PEG placement when clinically indicated. However, less than one third of them would consider tracheostomy when clinically indicated. Believers were more likely to reject invasive treatments than non-believers. Although rates of treatments acceptance were similar in HCP and ALS patients, HCP were more likely to change their advanced decision depending on their disability or quality of life. Most HCP recognized the importance of respecting patients' values, believes and autonomy on the decision-making process. The analysis of factors influencing the decision-making process in larger studies is warranted.

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## Exploring respiration and swallowing interaction by diaphragm motor evoked potentials in ALS patients

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Objective: Respiratory and swallowing disorders are the determining factors of prognosis in patient with amyotrophic lateral sclerosis (ALS). The interaction between this two basic vital functions and their relationship with survival were evaluated by diaphragm motor evoked potential (Dia-MEP) in patients with or without dysphagia and respiratory dysfunction.
Methods: Twenty-three patients with sporadic ALS patients and 15 healthy, age-matched control subjects participated in the study. ALS patients were followed up for 4 years after enrollment in the study. Pulmonary function tests were performed using forced vital capacity and maximal sniff nasal pressure (SNIP). Swallowing functions of patients were observed using the dysphagia limit. TMS was performed, recording by surface electrodes from bilateral hemidiaphragm, abductor pollicis brevis (APB) and tibialis anterior (TA). Latency of cortical and spinal motor-evoked potentials (Cx-MEP/Sp-MEP), MEP amplitude and central motor conduction time were measured.
Results: In patients with ALS, obtaining rate of Cx-MEP from APB, TA and diaphragm (Dia) muscles, and Sp-MEP from diaphragm were statistically significantly lower than control group. In patients with ALS, obtaining rates of Sp-MEP from APB and TA muscles were statistically significantly lower than CX-MEP. Cx-MEP was obtained in eight and 13 diaphragm muscles of patients with and without dysphagia, respectively. In patients with respiratory dysfunction, the obtaining rate of Dia Cx-MEP was statistically significantly lower.
Conclusions: Failure to obtain Dia-Cx-MEP in ALS patients pointed to the deterioration of corticodiaphragmatic pathway. Although there is a correlation between respiratory dysfunction and impaired swallowing, there is not a correlation between diaphragm MEP pathology and dysphagia and survive.

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## A diagnostic pathway in Polish patients with ALS

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting the upper (UMN) and lower motor neuron (LMN). Classified as a rare condition and mimicking more common diseases, ALS permanently causes diagnostic difficulties.
Objectives: To characterize the pre-diagnosis pathway undertaken by patients with ALS in the Polish healthcare system.
Patients and methods: We prospectively analyzed data of 88 ALS patients followed at the Neuromuscular Disorders outpatient clinic in Warsaw between 2015 and 2016. The study was based on data gathered from the 156-item questionnaire created in the frame of the ONWeBDUAL project (JPND). The study group included 39 male and 49 female patients, mean age 60.5 years, age of symptoms onset 55±30 years, disease duration - 28 months. Seventy seven percent of patients had a limb onset, 77% had a prevalence of the UMN over the LMN involvement. Fourty one percent of patients lived in villages or small towns, whereas 59% in larger towns and cities.
Results: The diagnosis delay was 19.2 months; a time-gap between the first medical observation and the ALS diagnosis was 14.8 months. The first specialist to consult a patient after the symptom onset was a neurologist (55.5%) or a general practitioner (31.8%). Orthopaedic surgeons (4.5%) and ENT specialists (3.4%) consulted ALS patients less frequently. The diagnosis was predominantly made by the 3rd physician (51.1%), less frequently by the 2nd (25%) or the 1st (6.5%), the majority (98.9%) being neurologists. The age, gender and place of living, as well as the UMN or LMN prevalence did not influence the diagnosis delay nor the consulting specialists order. The youngest patients, age<40 years, would consult a neurologist as their first choice physician more frequently than older individuals.
Conclusions: There is a need for supplementary education for health care providers to increase knowledge of early symptoms of ALS and to promote early referral to ALS diagnostic centers.

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## Assessment of the functional state of ALS patients in relation to physical activity

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Introduction: Amyotrophic lateral sclerosis (ALS) in the absence of effective treatment in a short time leads to deterioration of the functional state and reduced mobility.
Aim: The aim of the study was to evaluate the physical activity of patients with ALS and analysis of the relationship between muscle strength, functional status and physical activity of patients.
Material and methods: The study involved 76 ALS patients (F:37, M:39) followed between May 2013-October 2016 at the Department of Neurology, Medical University of Warsaw, Poland. Muscle strength (MRC), the functional state (ALSFRS-R) and the physical activity (questionnaire) were assessed in all patients every 3 months.
Results: There was a significant patients drop-out from the initial to the final visit (100%, 36.8%, 22.4%, 15.78% and 10.5%, every three months respectively). The ALSFRS-R fell down for the first 6 months (33.6, 32.1 and 22.4) while a pseudo-improvement was observed on a consecutive visit due to the drop-out of the most impaired patients. The physical activity questionnaire performed on the initial visit showed a high frequency of exercises in 57% patients with ALSFRS-R 40 (35%). Interestingly, 67% of bed-ridden patients would make everyday exercises compared to only 40% of mobile patients. 100% of the most functionally impaired patients performed longer exercise sessions (at least 20 minutes daily) as compared to 60% of mobile patients with average exercise time of 5 minutes.
Conclusions: Due to progressive muscle strenght and functional deterioration in the course of ALS, it is difficult to conduct longitudinal studies on rehabilitation efficacy in this group of patients. The functional state of ALS cohort deteriorates gradually in the course of disease and its interpretarion is altered by a significant patient drop-out. Unexpectedly, the loss of independent mobility and functional status does not prevent patients' physical activity.

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## ONWebDUALS: the European project funded by national agencies under the patronage of Joint Programme – Neurodegenerative Disease Research (JPND)

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Present understanding of the risks factors related to ALS is incomplete, presumably because they have never been integrated with phenotype-genotype patient profiles. The aim of the presented project is to define specific risk factors taking into account the genotype-phenotype background. In the frame of the project we have built a standardized patient questionnaire and an ALS domain ontology representing the body of medical knowledge related to this disorder. The ontology served as a formal basis for the construction of standardized E-health record of de-identified ALS patients, implemented in a European ALS web-database. The information on the possible risk factors included in the database will be analyzed to search for causal relationships between individual risk factors and ALS genotype-phenotype. The project started at March 1st, 2015. The preliminary version of patient questionnaire was accepted in June and its final version, in November 2015. The collection of patient and control data is continued since June 2015. In January 2016 Hilmi Uysal joined the team as a member of the consortium. Development of the database interface was completed in 2016. Testing preliminary version was performed by the project participants from the IBBE, where the servers hosting the database are localised. At the beginning of 2016 the electronic version of the questionnaire (MSExcel) was developed in IBBE. This ameliorated data transfer for database testing and enabled preliminary statistical analysis on the sample of about 500 patients. The web-database is now fully functional and incorporates data of patients and control subjects. The current number of patients is now around 900.
Conclusion: The project will be funded until the end of February 2018, but probably extended for a few more months. However, we hope that the research based on our database will continue beyond this date. First relevant results from this project will be shared with the ALS community soon.

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## Motor neuron disease: a clinical case with unexpected evolution

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We present the case of a woman, who came to our attention at the age of 56 because of a walking instability and scanning speech dysarthria, started three years before. Familiar history was negative for neurological disorders. Neurological examination showed mild ataxic-spastic walking, bilateral horizontal exhaustible nystagmus, mild dysmetria, scanning speech, brisk deep tendon reflexes, bilateral clonus. Brain MRI showed moderate enlargement of pericerebellar cerebrospinal fluid spaces. Genetic investigations for SCA (SCA1, SCA2, SCA6, SCA21), FA, KIF5A, HSP (SPG3A, SPG7, SPG31, SPG4, SPG5) mutations were negative. She was diagnosed as “ataxic-spastic syndrome” and follow up started. Afterwards the patient experimented a slow worsening of upper motor neuron signs, with increasing spasticity in walking, she also showed dysphagia and marks of spastic dysarthria, without modifications of cerebellar signs; so, at the age of 60, her disease was diagnosed as “primary lateral sclerosis plus”. She kept stable until she was 62 years old, when she presented progressive hyposthenia and fasciculations of the four limbs. Electromyography confirmed the presence of neurogenic damage with denervation and widespread fasciculations. The atypical evolution, with lower motor neuron involvement 10 years after the clinical onset, justified the execution of cerebrospinal fluid exam (that was normal) and treatment with intravenous immunoglobulins as a therapeutic attempt, without positive response. Eventually, the diagnosis of “amyotrophic lateral sclerosis” was made and therapy with riluzole was started. Genetic investigations for SOD1, FUS, TDP43 mutations were negative. During the first year after lower motor neuron involvement onset, the patient presented a drastic clinical worsening with muscular hypotrophy and four limbs distal hyposthenia; then, during the following 15 months (till nowadays) the clinical progression of lower motor neuron signs stopped. This clinical case confirm the extreme variability of presentation and evolution of a disease that, still nowadays, has got a wide range of different phenotypes despite the unique definition of motor neuron disease.

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## Age- and education-adjusted cut-off scores for the German parallel versions of the ECAS

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Background: The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is a well-validated and widely used tool to detect cognitive and behavioral impairments in patients with amyotrophic lateral sclerosis (ALS). It encompasses three ALS-specific (language, verbal fluency, executive function) and two ALS-non-specific (memory, visuospatial perception) domains. Two parallel versions (ECAS B&C) of the original ECAS have been developed to control for potential learning effects in longitudinal studies. Furthermore, previous research has shown that due to differences in cognitive capability the use of age- and education-adjusted cut-off scores is necessary for reliable assessment. The aim of this work therefore was to develop age- and education-adjusted cut-off scores for the German parallel versions of the ECAS.
Methods: In total, N=62 healthy participants underwent testing with ECAS B&C. They were subdivided into four groups according to their age and years of education: Group 1: <60 years of age and ≤12 years of education (N=11, 4 female) Group 2: 12 years of education (N=23, 8 female) Group 3: ≥60 years of age and ≤12 years of education (N=12, 9 female) Group 4: ≥60 years of age and >12 years of education (N=16, 4 female) Cut-off scores for each domain and the ECAS total score were calculated according to standard procedures, i.e. by subtracting twice the standard deviation from the mean of each group.
Results: In ECAS version B and C, younger participants performed better than older ones (B: $p<0.08$ C: $p<0.07$) and those with more education years performed significantly better than those with less (B and C $p<0.001$).
Discussion: This work highlights the fact that age and education have to be taken into account when assessing ALS patients' cognitive functions. It provides age- and education-adjusted cut-off scores for both parallel versions of the German ECAS for everyday clinical use.

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## Slovenian version of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

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Cognitive changes in patients with ALS often present as deficits in executive functions and changes in language and social cognition. In disease management, cognitive dysfunction may impair patient's decision-making ability. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) has been developed to detect the specific profile of cognition and behaviour changes in ALS and to differentiate it from other disorders (1). It is a 15-20 min screen that includes ALS-specific and nonspecific functions and a carer behaviour screen. We translated and adapted the original version of the screen. Using ECAS, cognitive status of 41 ALS patients (mean age 63.7, range 41-87 years) and 42 healthy controls (mean age 65.4, range 41-85 years) was evaluated. 32 carers completed the behavioural interview. Data from healthy controls was used to produce abnormality cut-offs. We tested the statistical correlation between demographic and clinical patient variables and ECAS scores. The comparison of the two groups revealed significant differences in all ECAS subdomains, with the exception of visuo-spatial functions. Twenty-four percent of patients scored below the cut-off in the ALS-specific domain and 27% for the ECAS total score. Behavioural changes were found in 32% of patients. Patients with bulbar onset of disease showed significantly more cognitive impairment than those with spinal onset. No correlation was found between cognitive impairment and age, education, duration, stage of disease or respiratory status. The results of the Slovenian version of ECAS are comparable to other published versions. ECAS is an effective and useful clinical tool that can improve the quality of patient care.

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## Premorbid neuropsychiatric disease in patients with motor neurone disease in Scotland

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Background: Cognitive and behavioural change are key features in up to 50% of patients with motor neurone disease (MND). A recent retrospective analysis indicated depression may be an independent risk factor for MND and an early indicator of neurodegeneration (1). The lifetime frequency of a mood disorder from UK Biobank data is 27%. We aimed to review the neuropsychiatric evolution of MND by assessing the frequency of premorbid neuropsychiatric disease in a prospective population-based Scottish cohort.
Methods: Incident patients diagnosed with MND in 2015 and 2016 were recruited via the Scottish Clinical Audit Research and Evaluation for MND (CARE-MND) platform (REC 15/SS/0216). Patient demographics, past medical and drug history, and disease progression data were collected using the CARE-MND national proforma. ICD-10 classifications were used to identify neuropsychiatric disease. Statistical analysis included association testing and logistic regression modelling.
Results: 284 patients were eligible for analysis: male-to-female ratio was 1.8:1, mean age of onset 64 years (95% CI 62.68-65.46). 58 (20%) of patients reported a history of ≥1 neuropsychiatric disease prior to onset of MND. Mood disorders predominated (40/284, 14%). 61 (22%) of patients had cognitive/behavioural change as part of the spectrum of disease of MND. Evidence of premorbid mood disorder or deliberate self-harm were significantly associated with MND-associated cognitive/behavioural change (p=0.004 and p=0.008 respectively). Conversely, history of psychotic illness and anxiety disorders were not associated with this phenotype (p=0.517 and p=0.277). On logistic regression modelling, history of mood disorder only predicted neuropsychiatric symptoms (p=0.033, OR 2.307 (95% CI 1.069-4.980)). 14 (5%) patients were documented to be taking psychotropic medication prior to MND onset. This was significantly associated with cognitive/behavioural change in MND (p=0.009) but was not included in the model due to its likely confounding effect.
Discussion: While our data suggest that premorbid mood disorder is less common in patients with MND compared with the UK Biobank population, our analysis supports the hypothesis that mood disorders predict cognitive/behavioural impairment in MND. This has implications for identification of pre-symptomatic biomarkers for MND.
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## The contribution of social cognition to social behaviour: cognitive predictors of behavioural change in ALS

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Introduction: ALS is a clinically heterogeneous neurodegenerative disorder associated with cognitive and behavioural impairment. The aim of the present study was to delineate the impact and relationship between cognitive dysfunction and behavioural change in ALS.
Methods: A cross-sectional population-based research design was applied to examine behavioural data from ALS patients (n=65) and healthy controls (n=66). Patients were screened for the C9orf72 repeat expansion. Patients completed a battery of neuropsychological tests, and the Beaumont Behavioural Inventory (BBI).
Results: Twenty-four patients had evidence of single- or multi-domain cognitive impairment (37%). Of those who were cognitively intact (n=41), 30% had evidence of mild-moderate behavioural change (n=12), with 7% reported to have severe behavioural impairment (n=3). Executive dysfunction was evidenced in those with behavioural impairment (p=0.001). A predictive model of performance on measures of executive function i.e., the Stroop task, verbal fluency index and Reading Mind in the Eyes Test, with the BBI as the outcome variable, was significant (p=0.011, C-statistic=0.794). A significant predictor of behavioural dysfunction in this model was social cognitive performance (p=.031), yielding an R2=.188, with the total battery yielding R2= .616.
Conclusion: These data show that while executive dysfunction is related to behavioural change, performance on measures of social cognition is most predictive. Assessment of social cognition can provide clinically relevant information for clinicians, and implications are discussed.

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## ATXN1 intermediate-length polyQ expansions are associated with C9orf72/ALS

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The genetic component plays a central role in the pathogenesis of both familial and sporadic amyotrophic lateral sclerosis (ALS). In addition to causative genes, several genetic variants have been described increasing the risk of developing ALS or acting as disease modifiers. Intermediate repeats in the polyQ of ATXN2 (29-33 CAG) are significantly associated with increased risk for ALS, while expansions greater than 34 cause spinocerebellar ataxia type 2 (SCA2). ATXN1 is another polyQ protein and alleles with more than 39 triplets cause spinocerebellar ataxia type 1 (SCA1). We investigated the role of ATXN1 in a cohort of 1212 Italian ALS patients and 529 healthy controls. All patients were also screened for variants in SOD1, TARDBP and FUS and for expansions in C9orf72 and ATXN2 genes. We detected ATXN1 alleles with intermediate length ≥33 polyQ repeats in 112/1212 patients (9.2%) and 29/529 controls (4.78%) (OR 1.63; CI 1.06-2.5; p= 0.016). When results were analyzed separately in the different groups of patients with or without variants in the examined genes we found that polyQ repeats were strongly enriched in ALS patients with the pathological C9orf72 expansion. 12/61 carriers of the C9orf72 expansion (19.6%) had at least one allele with a ≥33 polyQ repeats (p=0.0012; OR 4.2; CI 2.0268 to 8.7965). Considering the total of ALS patients with no variants in C9orf72, SOD1, TARDBP and FUS (n=1100), 96 (8.7%) had alleles with ≥33 repeats, a proportion that was still significantly increased compared to controls (p=0.025). In the C9orf72/ALS group no significant difference was observed between patients with and without CAG repeats ≥33 with respect to age of onset, site of onset, prevalence of FTD, survival and presence of familiarity. Our results strongly support the hypothesis that ATXN1 could act as a disease risk in C9orf72/ALS patients while its role in the other ALS subgroups of patients is less clear. Further studies are needed to confirm our results and to define the mechanism by which ATXN1 might contribute to neuronal degeneration leading to ALS.

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## Novel UBQLN2 mutations linked to amyotrophic lateral sclerosis and spastic paraplegia through defective proteolysis

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Ubiquilin-2 regulates several protein degradation pathways including the ubiquitin-proteasome system (UPS), the endoplasmic reticulum-associated protein degradation (ERAD) pathway and the macroautophagy. Defects in these processes could contribute to accumulation of aggregated and/or misfolded proteins in amyotrophic lateral sclerosis (ALS) disease, as ubiquilin-2 is a component of the ubiquitin inclusions detected in degenerating motor neurons in ALS patients. Mutations in UBQLN2 have been associated with X-linked juvenile and adult forms of ALS and ALS linked to frontotemporal dementia (FTD). We performed genetic analysis of 400 familial (FALS) and 770 sporadic (SALS) cases and identified three novel mutations in the PXX repeat domain of UBQLN2, a hot spot domain for ALS/FTD mutations. One of these mutations was also identified in patients with spastic paraplegia. These mutations were predicted to be deleterious by SIFT in silico analysis and were absent from ExAC and gnomAD databases. Patient lymphoblasts carrying a UBQLN2 mutation showed (i) absence of ubiquilin-2 accumulation and (ii) modified proteolysis pathways. Our results confirm the role of PXX repeat in ALS pathogenesis, expand the clinical spectrum of UBQLN2 mutations to spastic paraplegia phenotype, evidence a highly reduced disease penetrance in females carrying UBQLN2 mutations, which is an important information for genetic counseling, and underline the pivotal role of ubiquilin-2 in proteolysis regulation pathways.

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## TBCE mutations cause early-onset progressive encephalopathy with distal spinal muscular atrophy

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Tubulinopathies constitute a family of neurodevelopmental and neurodegenerative disorders caused by mutations in several genes encoding tubulin isoforms. Loss-of-function mutations in TBCE, encoding one of the five tubulin-specific chaperones involved in tubulin folding and polymerization, cause two rare neurodevelopmental syndromes, hypoparathyroidism-retardation-dysmorphism and Kenny-Caffey syndrome. While a missense mutation in TBCE has been associated with progressive distal motor neuronopathy in the pmn/pmn mice, no similar degenerative phenotype has been recognized in humans. We report on the identification of an early-onset and progressive neurodegenerative encephalopathy with distal spinal muscular atrophy resembling the phenotype of pmn/pmn mice and caused by biallelic TBCE mutations, with the c.464T>A (p.Ile155Asn) change occurring at the heterozygous/homozygous state in 6 affected subjects from 4 unrelated families originated from the same geographical area in Southern Italy.

Western blot analysis documented a reduced amount of TBCE, suggestive of rapid degradation of the mutant protein, similarly to what was observed in pmn/pmn mice. The impact of TBCE mutations on microtubule polymerization was determined using biochemical fractionation, and analyzing the nucleation and growth of microtubules at the centrosome and extracentrosomal sites after treatment with nocodazole. Primary fibroblasts obtained from affected subjects displayed a reduced level of polymerized α-tubulin, similarly to tail fibroblasts of pmn/pmn mice. Moreover, markedly delayed microtubule re-polymerization and abnormal mitotic spindles with disorganized microtubule arrangement were also documented. While loss-of-function of TBCE has been documented to impact on multiple developmental processes, the present findings provide evidence that hypomorphic TBCE mutations primarily drive neurodegeneration.

**P57**

## New FIG4 gene mutation causing fast progressing ALS phenotype: a case report

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FIG4 gene mutations cause several neurodegenerative syndromes including Charcot-Marie-Tooth disease type 4J and, rarely, sporadic and familiar amyotrophic lateral sclerosis (ALS) with autosomal dominant transmission. A 27-years old woman was referred for rigidity in lower limbs associated with progressive verbal fluency impairment and dysphagia lasting for over one year. She was adopted and family history was unknown. Past medical history was characterized by moderate cognitive retardation known from the early infancy without motor deficits. As the patient came to our attention (May 2016), she presented severe motor aphasia and spastic paraplegia. Mild proximal muscular weakness was identified in lower limbs together with fasciculations. Diffuse severe spastic hypertonia with brisk reflexes and bilateral Babinski sign was present. At cranial nerves examination, a long-lasting multi-directional non-positional nystagmus was observed. Blood and cerebrospinal fluid analysis were normal as well as EMG and nerve conduction studies. Brain MRI showed diffuse cortical atrophy more evident in the temporal lobes and mild atrophy of the corpus callosum. Muscle biopsy showed initial signs of neurogenic atrophy. A diagnosis of juvenile ALS was made and genetic screening for SOD1, FUS, TDP43 and C9Orf72 performed, which was negative. Extended genetic analysis for ALS-related genes was carried out, which evidenced a composed heterozygous mutation in the FIG4 gene (c.122T>C and c.1667C>T). The first mutation is known for causing upper-motor neuron dominant (UMND) ALS. The other was never described before in humans but in silico studies demonstrate a probable pathogenic role in ALS. Later, symptoms rapidly worsened to a spastic tetraplegia associated with complete aphasia and severe dysphagia. The patient died after ab ingestis pneumonia 18 months after symptom onset. FIG4 mutations usually determine slow progressive UMND ALS phenotypes with onset in the adult age. Here we describe a novel composed mutation in the FIG4 gene causing a juvenile fast progressive form of ALS associated with severe brain involvement. This report widens the spectrum of FIG4 mutations and describes an unexpected, novel, early onset and quickly progressing phenotype. Further studies analysing complete pedigrees are warranted to better understand mechanisms underlying pathogenesis and phenotype variability in FIG4-related diseases.

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## A retrospective analysis of the genotype-phenotype relationships in familial MND and MND-FTD within a South London population

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Introduction: Approximately 10% of MND cases have a family history of MND/MND-FTD, of which 60% can be attributed to a genetic mutation in one of at least 24 genes. The most commonly affected genes are C9ORF72, SOD1, FUS and TARDBP. In total, 23 subsets of MND/MND-FTD have been described. Although variations in clinical parameters between the subsets exist, significant overlap remains. In our pursuit of novel affected genes since 2000, we have collected and analysed 177 blood samples from patients with familial MND/MND-FTD attending the King's College Hospital Motor Nerve Clinic. We have reviewed the clinical details of this cohort.
Methods: Clinical information has been retrospectively sourced from patient records and collated with our extensive genetic database. Genetic samples have undergone a mixture of exome and Sanger sequencing. Statistical analyses were carried out in SPSS and involved Chi-squared, ANOVA and Pearson's correlation testing.
Results: A total of 177 cases were analysed. The following causative genes were identified: C9ORF72 expansion (20% of cases), SOD1 (13%), FUS (5%), TARDBP (2%), with <1% for HNRNPA1, UBQLN2, ANXA11, PFN1, MATR3 and TBK1. Half of patients continue to have no identifiable causative gene.
Findings of note include: The mean age of onset was significantly higher for the C9ORF72 group (59 years) than the SOD1 group (46 years; $p<0.05$). The male:female ratio was reversed for the SOD1 group (1:1.3, compared to 1.2:1 overall; NS, $p=0.2$). An upper limb presentation was more frequent (NS) in patients with C9ORF72 expansions (8/11) and SOD1 (7/10). All of the TARDBP (n=2), UBQLN2 (n=1) and PFN1 (n=1) cases presented with lower limb symptoms. There was a trend for shorter diagnostic delay for patients carrying the C9ORF72 expansion, SOD1 and TARDBP mutations, compared to the undetermined group. In contrast, the trend was for longer diagnostic delay for the FUS group. There were 22 definite and 11 possible cases of MND-FTD. Of the definite cases, 14 were associated with the C9ORF72 expansion ($p<0.05$). Age of onset was negatively correlated with diagnostic delay ($r=-0.36$, $p<0.05$).
Conclusions: We have updated and extended the clinical information available in our expanding genetic database of MND and MND-FTD cases. The statistically significant results are consistent with published genotype-phenotype relationships. We aim to repeat the analysis as we obtain more cases.

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## Intermediate CAG repeats in the ATXN2 gene in patients with amyotrophic lateral sclerosis from a Brazilian Research Center

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Background: Genetic mutations are defined risk factors for ALS up to date. Intermediate expansions of CAG repeat (27 – 33 repeats) in ATXN2, the causative gene of spinocerebellar ataxia type 2, have been associated with an increased risk for amyotrophic lateral sclerosis (ALS) in different populations. This study investigated the presence of increased ATXN2 CAG repeats in a cohort of Brazilian patients clinically diagnosed with ALS.
Patients and methods: The CAG repeat length in the exon 1 of ATXN2 was assessed in 240 sporadic and 48 familial ALS cases as well as 279 controls using fragment analysis with fluorescently labeled primers. PCR products were separated by capillary electrophoresis using an ABI 3730. CAG repeat length was determined using the GeneMarker software version 2.6.4 (SoftGenetics).
Results: Most Brazilian ALS cases and controls were homozygous for 22/22 or 23/23 repeats or heterozygous for 22/23 repeats of the ATXN2 gene. ATXN2 intermediate length repeats alleles (≥27) were observed in nine sporadic ALS patients (3.75%), one familial ALS patients (2.08%) while being observed in two controls (0.71%), (p= 0.017 and p= 0.36, respectively). ATXN2 CAG intermediate length repeats were 27, 28, 29, 30, 32, 33 in n=1, 3, 3, 1, 1, 1 ALS subjects, respectively, and 27 in n=2, control subjects, respectively.
Conclusion: Increased frequency of ATXN2 intermediate expansion was detected in this partial report of an ALS sample of ALS Unit of University of São Paulo Medical School.
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## SOD1, TDP-43, FUS/TLS and C9orf72 genes in Serbian ALS patients: long term survey

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Amyotrophic lateral sclerosis (ALS) is a genetically heterogeneous neurodegenerative disorder associated with a progressive neuronal loss and degeneration of motor neurons in the cerebral cortex, brainstem and spinal cord. Signs of frontotemporal dementia or milder cognitive deficits may also be present. Approximately 5% to 10% of ALS patients have positive family history, mostly with autosomal dominant inheritance. Since major outbreak, with discovery of SOD1 gene mutations in ALS patients, there is a growing list of genes that can be attributed to ALS, among them TDP-43, FUS/TLS and C9orf72, along with SOD1 as most prominent. From discovery of C9orf72 expansions, lot of genetic research data points out C9orf72, as a probable major causative gene in ALS. Here we present results of long term genetic survey of SOD1, TDP-43, FUS/TLS and C9orf72 genes in 431 Serbian ALS patients (49 familial and 382 sporadic) who referred to the Neurology Clinic in Clinical Center of Serbia from 1999 till 2016. year. Overall, 80 (18,5%) of all ALS patients carried mutations in tested genes – 42/49 (85,7%) FALS patients and 38/382 (9,94%) SALS. Mutation L144F in SOD1 gene was the most common (31/49 and 15/382) followed by expansion of hexanucleotide GGGGCC repeats in C9orf72 gene (6/49 and 9/382). One patient, along with C9orf72 expansion, carried also mutation in FUS/TLS gene (H517P). Further, in 8/382 SALS patients we observed D90A, and in 4/49 FALS A145G mutation in SOD1. As a part of this survey, we also identified 3 mutations for the first time, all in SOD1 gene (A145G, IVS2+1G>A and P66S). Two mutations in TDP-43 gene were found - M337V in one FALS and G384R in one SALS patient. Mutations in FUS/TLS gene were observed in 3 SALS patients (R512C in two and R521H). As a conclusion, we can underline SOD1 gene as still the major causative gene in Serbian ALS patients, which, along with expansion in C9orf72 gene, can explain foundation of the disease in majority of Serbian FALS patients. On the other side, there is still a big lack of knowledge in SALS patients.

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## Neuroimaging patterns along the ALS-FTD spectrum: a multiparametric imaging study

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Background: Frontotemporal dementia is associated with considerable clinical, genetic and pathological heterogeneity. The objective of this study is to characterise the imaging signatures of the main FTD phenotypes along the ALS-FTD spectrum.
Methods: A total of 100 participants underwent comprehensive multimodal neuroimaging, genetic testing and neuropsychological evaluation. Seven patients with behavioural variant FTD (bvFTD), eleven patients with non-fluent-variant primary progressive aphasia (nfvPPA), two patients with sematic-variant primary progressive aphasia (svPPA), twenty patients with amyotrophic lateral sclerosis and FTD (ALS-FTD), twenty ALS patients without behavioural or cognitive deficits (ALSnci) and forty healthy controls (HC) were included in a prospective quantitative neuroimaging study.
Results: Phenotype-specific spatial patterns of pathology were identified along the ALS-FTD spectrum, highlighting a strikingly focal distribution of disease-burden as opposed to global atrophy. Significant motor cortex and corticospinal tract degeneration was identified in both bvFTD and nfvPPA patients. ALS-FTD patients exhibited widespread extra-motor pathology and significant precentral gyrus atrophy compared to ALSnci patients. ROI analyses confirmed focal grey matter alterations in Broca's and Wernicke's area in language variant FTD cohorts.
Conclusions: Our findings confirm that the clinical manifestations of FTD are underpinned by phenotype-specific patterns of white and grey matter degeneration.

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## A 18FDG-PET study on ApoE genotype in ALS

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Background: In Alzheimer's Disease (AD) the presence of the ε2 allele of ApoE lowers the risk and delays the onset of the disease, while the ε4 isoform increases the risk of dementia by 3-fold in heterozygous carriers and 12-fold in homozygous carriers. Conversely, we recently found in a population-based series of ALS patients, collected through the Piemonte and Valle d'Aosta Register for ALS, that ε2 allele provides an increased risk of FTD in ALS patients. The aim of the present study was to evaluate the metabolic correlates of the ApoE genotype in ALS patients.
Methods: The ApoE genotype (from ε2/ε2 to ε4/ε3) was regressed in 159 ALS patients against whole brain metabolism as assessed by 18FDG-PET. SPM8 Multiple Regression routine was implemented with age, sex, education and type of onset as covariates. Statistical significance threshold was set at $p<0.005$ uncorrected.
Results: Higher metabolism positively correlated with genotype lacking ε2 alleles in correspondence of bilateral frontal, prefrontal, orbitofrontal and anterior cingulate cortices as well as in the right thalamus. No significant negative correlation was found.
Conclusion: The metabolic correlate of the presence of ε2 alleles in ALS patients is a lower 18FDG uptake in brain areas tipically affected when a comorbid cognitive impairment is present. These data strengthen our previous finding of a role of the ε2 isoform of ApoE in increasing the risk of frontal cognitive deficits in patients suffering from motor neuron disease.

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## Myelin imaging in amyotrophic lateral sclerosis: a comparison with multiple sclerosis using quantitative magnetisation transfer

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Background: Recent evidence suggests that oligodendrocyte dysfunction and myelin damage may play a key role in the complex processes involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). Consequently, there has been a growing interest in exploring the degree and pattern of myelin loss in ALS and how this relates to axonal loss. Emerging imaging techniques enable further exploration of this question.
Objectives: To compare myelin changes in the motor system in ALS patients to healthy controls and to patients with multiple sclerosis (MS), the latter being a prototypical myelin disorder.
Methods: The participants underwent quantitative magnetization transfer (qMT) magnetic resonance imaging (1.5 Tesla) using the balanced steady-state free precession (bSSFP) method. Parameters obtained included the ratio of restricted to free pool size (F) which closely correlates with myelin content in the white matter; and the forward exchange rate (kf) which seems to correlate most closely with metabolic abnormalities.
Results: The study included 21 ALS patients, 39 MS patients and 19 controls. On comparing F in motor cortex and corticospinal tract (CST) in in ALS patients to controls, significant reductions were observed in the left cerebral peduncles though differences did not survive correction for multiple comparisons. Altough F was signifcantly reduced in MS patients compared to ALS patient, the difference reached statistical signifcance only in the rostral portion of the CST. ALS patients demonstrated significant reductions kf compared to controls in the left corona radiata and bilaterally in the posterior limb of the internal capsule but not in the motor cortex.
Conclusion: This exploratory study provides preliminary data suggesting that myelin loss and metabolic disturbances in ALS emerge in the distal regions of the motor system in with subsequent spread rostrally. A larger study which includes spinal cord imaging would be a useful step in investigating this possibility.

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## The role of iron-related hypointensities on brain MRI as a biomarker in amyotrophic lateral sclerosis

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Introduction: Iron related hypointensities in the motor cortex (IRhMC) have been described on T2-weighted MRI in amyotrophic lateral sclerosis (ALS) patients, but also in healthy subjects. Factors determining these changes have been insufficiently studied and its meaning remains controversial. In this study, we evaluated the contribution of demographical, clinical, analytical and genetic factors to IRhMC in both patients and controls and assessed the diagnostic validity of this sign.
Methods: One hundred and two ALS patients and 48 controls were included in the study. Susceptibility-weighted images (SWI) were obtained on a 3T MRI. Two experienced radiologists, blinded to the clinical status of subjects, visually assessed the images independently, qualitatively scored the IRhMC and, based on this score, classified subjects as patients or controls. Age, gender, family history, demographical and clinical variables were recorded. Baseline levels of ferritin were measured in patients. The C9ORF72 expansion was studied in all patients and the SOD1 in familial ALS patients who did not carried the expansion.
Results: The intraobserver agreement for the score was good (0.77 [0.73, 0.82]) and very good for the final diagnosis (K=0.86 [0.78, 0.95]). Sensitivity, specificity and diagnostic accuracy in distinguishing patients and controls based on the IRhMC were 73%, 91% and 81% respectively. In controls, IRhMC was associated with age. In ALS patients, IRhMC was associated with premorbid body mass index, UMN impairment signs and bulbar onset, but not with other demographical, clinical or biological factors. No differences were found between genetic and sporadic patients.

Moreover, the intensity and extent of IRhMC in the different regions of the motor homunculus were associated to the site of symptoms onset.
Conclusions: IRhMC shows good specificity but poor sensitivity for ALS diagnosis. Moreover, it is a reliable marker of UMN degeneration in ALS patients and is more frequent in bulbar onset patients. Age and premorbid body mass index should be considered as possible modifiers of the IRhMC. However, other clinical, biological and genetic factors show little influence. The regional analysis of the IRhMC following the motor homunculus could be an easy and reliable method to quantify and monitor the UMN loss.

**P73**

## Synaptotagmin 13 protects motor neurons from degeneration in ALS

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Mechanisms responsible for motor neuron (MN) subtype-selective degeneration in amyotrophic lateral sclerosis (ALS) remain largely unknown. However, the molecular signature of degeneration-resistant oculomotor neurons (OMNs) is distinct from that of vulnerable spinal, cortical and lower brainstem MNs, thus offering some clues to differential vulnerability. We here demonstrate that OMNs show preferential expression of synaptotagmin 13 (SYT13) and that expression is maintained in OMNs and remaining spinal MNs in end-stage ALS patient tissues, suggesting a role in their relative resistance. Overexpression of SYT13 in human in ALS in vitro models improves MN survival and increases motor axon length. Adeno-associated virus-mediated delivery of Syt13 to transgenic ALS mouse model improves pathology, delays muscle denervation and prolongs survival. Mechanistically, an increase in SYT13 reduces endoplasmatic reticulum stress and apoptotic signs. These findings sustain a role of SYT13 as a disease modifier and candidate therapeutic target for ALS. Our study demonstrates that exploring differential neuronal vulnerability may lead to new therapeutic strategies to prevent the progressive degeneration in ALS, but also in other MN diseases.

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## Establishing a patient-derived organoid model for studying cortical thinning in ALS

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Cortical thinning and gliosis are hallmarks of amyotrophic lateral sclerosis (ALS). Glial function and pro-inflammatory signatures are altered in ALS patients and brain regions with higher glial activation display decreased cortical thickness in ALS patients. Hexanucleotide (GGGGCC) repeat expansions in C9ORF72 are the most common cause of ALS and frontotemporal dementia (FTD; C9ALS/FTD). Imaging studies show that C9ALS patients present broad structural brain changes and cortical thinning outside primary motor areas. Furthermore, the presence of systemic immune deficiencies in C9ORF72 knockout (KO) mouse models and detection of glia pathology in C9ALS patients suggests a cell-autonomous role of C9ORF72 in glial cells. The molecular mechanisms underlying cortical thinning and its relation to glial dysfunction remain poorly understood. To further understand the contribution of C9ORF72 in cortical degeneration we aim to establish a patient-derived organoid model for cortical thinning. We differentiate both cortical and cerebral organoid models from control and ALS patient-derived induced-pluripotent stem cells (iPSC). Here, we present a characterization of the models and an overview of future experiments to dissect the role of C9ORF72 in ALS pathology and the development of therapeutic applications. Analysis of cortical layer development in both models reveals the presence of markers for both superficial and deep cortical layers. Furthermore, in the cerebral organoid model, different types of glial cells were identified, as were glia-neuron interactions. To allow 3D imaging of both cortical and cerebral organoids tissue clearing and light sheet microscopy approaches are being developed as part of the project. Comparison of organoids from control and C9ALS patients will reveal whether these models can eventually be used for studying cortical thinning.

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## Stress granules formation upon condition of chronic stress in human ALS fibroblasts

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Abnormal cytoplasmic aggregates of TDP-43 protein represent an hallmark of ALS and FTD diseases, as pathological inclusions of this protein were found in autoptic brain tissues from familial and sporadic ALS and FTD patients. The RNA-binding protein TDP-43 is also an important component of stress granules (SG), reversible and dynamic cytoplasmic protein/RNA complexes which form a response to environmental stress conditions, as demonstrated for the first time by our laboratory. Recently, SGs have been hypothesized to contribute to neurodegeneration in ALS/FTD via gain or loss-of-function mechanisms. In particular, pathological inclusions containing TDP-43 are supposed to derive from SGs that, in condition of a prolonged stress as it occurs during the neurodegenerative process, fail to be properly disassembled and, by persisting in the cell, eventually interfere also with the autophagic pathway. To better investigate this hypothesis we reproduced a status of chronic stress in vitro to evaluate if SGs are able to form in this condition and not only under sub-lethal environmental insults as described in literature so far. We used primary fibroblasts obtained from skin biopsies of healthy controls and ALS patients, which were exposed to low doses (5-50uM) of sodium arsenite for a prolonged time-course (1-6 days). We observed SGs formation during chronic arsenite treatment both in control and patients fibroblasts and, in comparison to SGs forming upon acute arsenite stress (0.5 mM for 30 minutes), they were significantly larger in size as assessed by image analysis. When we used fibroblasts derived from TARDBP and C9ORF72 ALS patients we also found differences in SGs formation as regards both number and size in a mutant gene-dependent manner. Our findings demonstrate for the first time that SGs may form not only upon sub-lethal environmental stress but, importantly, also in condition of chronic and prolonged insults in human patients cells. Therefore our data seem to support the hypothesis that SGs may indeed represent an initial response to oxidative stress and that they may then be converted into pathological inclusions contributing to neurodegeneration in ALS and FTD.

**P79**

## Molecular characterization of mouse optineurin insufficiency models

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Optineurin is a multifunctional ubiquitin-binding protein that acts as an adaptor in a variety of cellular processes including inflammatory signaling, apoptosis, necroptosis, autophagy, and vesicle trafficking. Optineurin mutations, as well as mutations in several optineurin-interacting proteins, such as TANK-binding kinase (TBK1) and p62/SQSTM-1, were recently found in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The majority of thus far characterized optineurin ALS mutations suggest that it causes disease by loss of function. Several models of optineurin insufficiency and deficiency were recently generated to assess the neuroprotective function(s) of optineurin in vivo. Here we compare two mouse models of optineurin insufficiency: OptnΔ157, in which the N-terminal TBK1-binding domain is deleted, and Optn470T, lacking the C-terminal ubiquitin-binding region. We argue that both the N-terminal and the ubiquitin-binding regions of optineurin are needed for TBK1 recruitment and subsequent activation. However, the functional losses in several optineurin-mediated processes in Optn470T and OptnΔ157 are usually subtle, rather than striking. We propose that the unique location of optineurin at the crossroads of several neuroprotective pathways allows the amplification of pathogenic stimuli, which could ultimately trigger neurodegeneration.

**P81**

## Implication of peripheral macrophages in amyotrophic lateral sclerosis

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Previous studies have highlighted the implication of microglial cells/macrophages in ALS disease progression without, however discriminating the role of the two populations. Motor neurons are specific neurons since their axon extends at the periphery and is therefore surrounded by peripheral macrophages while their cell body, is surrounded by microglia, the macrophages of the CNS. Although microglial cells and peripheral macrophages share common characteristics including phagocytic capacities or several markers, both populations have different developmental origins and are localised in different cellular environments which could lead to specific implication during the disease. Since previous reports showed an early degeneration of motor neuron axons and macrophages at the periphery would be more easily accessible than microglia in the CNS, we studied the potential of peripheral macrophages as therapeutic targets in ALS mice. First, we showed a strong and progressive macrophage activation in the sciatic nerve of SOD1G93A ALS mice suggesting an active role of macrophages in the pathology. We then developed a protocol (using a chemotherapy agent and bone marrow transplant) to replace macrophages at the periphery without affecting microglia to understand the role of macrophages on their own in the pathogenesis of ALS. We replaced mutant SOD1-expressing macrophages by control (GFP+) macrophages or macrophages with more trophic or less toxic potentials. Our protocol allowed an efficient replacement of monocytes/ macrophages (GFP+) in the blood and in peripheral tissues affected in ALS (sciatic nerve and gastrocnemius muscle). Importantly, GFP+ peripheral cells were only scarcely and transiently found in the spinal cord of grafted ALS mice showing that infiltration of peripheral monocytes/macrophages in the CNS is minor and that the effect of cell replacement, on the disease course, would come from the periphery. Replacement of mutated macrophages by cells more trophic (overexpressing hSOD1WT) or less toxic (KO for the superoxide producing Nox2) led to a decreased microglial activation in the spinal cord of ALS grafted mice, an increased motor neuron number at disease end-stage for the NOX2 KO-grafted mice, and a delay in reaching the symptomatic stage of the disease. In conclusion, we provide new evidences suggesting an active role of peripheral macrophages in the pathogenesis of ALS, supporting new therapeutic strategies by targeting peripheral macrophages.

P83

## Mutant superoxide dismutase aggregates from human ALS spinal cord transmit templated aggregation and fatal ALS disease in mice

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Mutations in superoxide dismutase-1 (SOD1) are a common known cause of amyotrophic lateral sclerosis (ALS). ALS patients and transgenic model mice carrying mutant human SOD1 (hSOD1) develop aggregates of the protein in motor neurons. In transgenic mice two strains of aggregates (denoted A and B) can arise. Inoculation of minute amounts of A or B aggregates into spinal cords of asymptomatic hSOD1 transgenic mice initiated spreading, exponentially growing templated hSOD1 aggregations concomitantly with premature fatal ALS. Here we explored whether prion-competent mutant hSOD1 aggregates also exist in human ALS. Aggregate seeds were prepared from spinal cords from a patient and transgenic mice carrying the hSOD1G127Gfs*7 mutation by centrifugation through density cushions. G127Gfs*7 mutant hSOD1 has a 26 amino acids long C-terminal truncation, but the core structure of the aggregates was strain A-like. Inoculation of the seeds into lumbar spinal cord of hSOD1-expressing mice induced strain A hSOD1 aggregation propagating along the neuraxis and fulminant fatal ALS. The potencies of the human-derived seed preparations were high and disease was initiated under conditions plausible to exist in human motor areas. Human and murine control seeds had no effect. Our results suggest that prion-like templated spread of hSOD1 aggregation could be the primary pathogenic mechanism, not only in hSOD1 transgenic models, but also in hSOD1-induced ALS in man.

**P85**

## Axonal transcriptome of stem cell-derived motor neurons in health and ALS

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Motor neurons are highly polarized cells. Their somas and associated dendrites are located in the brainstem and spinal cord, while their axons traverse large distances in the body and connect to muscle via specialized synapses termed neuromuscular junctions (NMJs). Motor axons and NMJs are primary targets in amyotrophic lateral sclerosis (ALS). Muscle denervation and axonal retraction commence before motor neuron somas in the spinal cord are lost. The presence of ribosomes in axons indicates local protein translation, however the axonal RNA composition is largely unknown. We aimed to screen the RNA content of motor axons and somas in health and ALS by differentiating mouse embryonic stem cells (mESCs) into spinal motor neurons. We used mESCs overexpressing the mutant human superoxide dismutase 1 (SOD1G93A) gene to model ALS. The motor neurons were cultured in microfluidic devices, which allowed spatial separation of the motor neuron axons and somas. Deep RNA sequencing was performed on both the axonal and somatodendritic compartments to investigate local RNA composition. We identified the axonal transcriptome, with >5000 transcripts detected in motor neuron axons, of which around 10% were enriched in the axonal compared to the somatodendritic compartment. Moverover, we observed alterations in the localization of several transcripts in SOD1G93A motor axons versus wild-type controls.

**P87**

## Evaluating the interdependence of misfolded SOD1 species in ALS pathogenesis

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ALS disease is a fatal neurodegenerative disease characterized by the selective loss of motor neurons. To this day, there is no cure or effective treatment for ALS. The development of effective therapies is severely hindered by our incomplete understanding of disease pathogenesis. A portion of familial ALS results from mutations in the ubiquitously expressed enzyme copper/zinc superoxide dismutase (SOD1). Most of these mutations introduce subtle conformational changes that yield "misfolded" SOD1 protein. However, the nature of this toxic misfolded protein is poorly understood. The ALS research community largely agrees that misfolded SOD1 lies at the root of toxicity. It is our hypothesis that multiple forms of misfolded SOD1 exist and demonstrate variable localization, cellular targets and/or potencies. Using a series of conformation-specific SOD1 antibodies, data is emerging that the term "misfolded SOD1" might encompass more than one form. Indeed, the Vande Velde team's published work using several of these antibodies distinguishes at least two types of misfolded SOD1. It remains unexplored whether these alternative forms of misfolded SOD1 exist as part of a continuum (implying interdependency) or arise independently. By extension, it remains unknown whether neutralization of one form will translate into an abundance or paucity of other forms. Whether these different forms of misfolded SOD1 are related to one another is now a central question. Using SOD1G93A mice, infused with antibodies that selectively recognize and neutralize specific misfolded SOD1 conformers, the proposed project will determine if a relationship exists between these structures. Importantly, these experiments will tease out the relationship between misfolded SOD1 conformers in vivo. They will provide critical information about the relative accumulation of misfolded SOD1 conformers in a therapeutic setting aimed to neutralize a specific misfolded SOD1 structure and further define the role of various non-native SOD1 conformers in disease initiation. This project will be informative to the ongoing development of these reagents as a therapy for ALS patients.

**P89**

## Translating ribosome affinity purification from C9orf72- ALS/FTD patient-derived iPS motor neurons

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Background: The intron 1 C9orf72 hexanucleotide repeat expansion is the most common cause of amyotrophic lateral sclerosis (ALS) in both sporadic and familial patients. Transcriptomic analysis of patient-derived iPS motor neurons is challenging due to multiple sources of experimental variance, including the presence of up to 30% of cells in culture being of unknown or non-neuronal identity.
Aims: The aim of this project is to use translating ribosome affinity purification (TRAP) to allow selective isolation of RNA from actively translating polysomes in a pure population of motor neurons to achieve a more accurate analysis of the translatome profile of this disease model. This will allow a more accurate estimation of the effect the C9orf72 repeat expansion has on gene expression in C9/ALS motor neurons.
Methods: Initially in the project, we generated a TRAP construct containing a major large ribosomal subunit protein, RPL22, fused with a FLAG affinity tag, and bicistronic enhanced green fluorescent protein (eGFP) gene, under the control of a choline acetyl transferase (ChAT) promoter. Magnetic beads coated with anti-FLAG antibodies will be used to capture FLAG-tagged RPL22 subunit-containing polysomes from iPS motor neuron lysates and standard RNA extraction will be used to purify motor neuron-specific mRNA from C9/ALS patient lines and CRISPR/Cas9-edited patient control lines. Following quality control steps, RNA will be sent for sequencing to identify key pathways associated with early changes of neurodegeneration in C9orf72 motor neurons.
Results: Molecular cloning was used to generate the TRAP lentivector, and lentiviral particles were generated via HEK293-T cell transfection. Confocal imaging of fixed control and patient iPS motor neurons transduced with ChAT:TRAP lentiviral particles has shown successful expression of the eGFP reporter and co-stained with ChAT and SMI-32 (mature motor neuron markers). Furthermore, preliminary western blotting has shown the expression of both endogenous RPL22 and exogenous RPL22-FLAG in iPS motor neurons.
Conclusions: In current work we are optimising the transduction conditions and RNA extraction methods from iPS motor neuron cultures in preparation for submission of samples for sequencing. Analysis of the C9/ALS patient translatome, in conjunction with proteomic analysis is anticipated to overcome some of the problems currently associated with the variation in transcriptomic outputs from iPSC models in ALS.

P91

## The capacity to maintain stress granule assembly is impaired by a preceding chronic stress – the "first hit" can sensitise neurons to the "second hit"

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Timely assembly and disassembly of stress granules (SGs) is an important mechanism of cell survival in the conditions of acute stress. Various acute stresses can play a role of the "second hit" triggering progression of a dormant neurodegenerative process in neurons already affected by a "first hit", e.g. malfunction of disease-associated proteins. Therefore, aberrant SG response is believed to contribute to the development of neurodegenerative changes in a wide range of conditions, including amyotrophic lateral sclerosis (ALS). Phosphorylation of eIF2alpha is a key regulator of SG assembly and disassembly, and increased levels of p-eIF2alpha are often observed in neurons of patients with neurodegenerative diseases as well as in various models of neurodegeneration. It is also known that pathological protein aggregation can induce some aspects of stress response in affected neurons without triggering of all-out stress response characterised by translational arrest and SG formation. Using several model systems, including ES cell-derived human neurons and live mice, we have demonstrated that elevated p-eIF2alpha level in cells experiencing transient or persistent mild stress impairs the maintenance of SG assembly following acute severe stress, creating a situation of SG partial loss-of-function. Upregulation of PP1 phosphatase regulatory subunits has been identify as a mechanism behind it. Results of histopathological analysis of ALS patients spinal cord sections were consistent with the results obtained in the model systems. We hypothesise that changes of neuronal physiology instigated by genetic or environmental factors can be tolerated for a long time but still cause persistent mild stress in affected neurons, preconditioning them to a defective and therefore deleterious response to any kind of acute, SG-inducing stress. This suggests that simultaneous moderation of PP1 phosphatase activity and p-eIF2alpha level might be considered as a therapeutic approach preventing progression of ALS pathology.

**P93**

## The homeoprotein Engrailed I in spinal motor neuron survival

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Homeoproteins (HPs) regulate gene expression during development and in the adult. In parallel with their cell autonomous activities, HPs can transfer between cells to regulate transcription and translation in a non-cell autonomous manner. In addition to their role in brain development, Engrailed-1 and-2 homeoproteins (En1/2) promote the survival of adult dopaminergic cells in several models of Parkinson's disease, and Otx2 promotes the survival of adult retinal ganglion cells in a mouse model of glaucoma. This protective activity is cell autonomous or non-cell autonomous. In ALS, motor neurons (MNs) degenerate resulting in muscle weakness and death with a rapid disease progression. In the spinal cord En1 is expressed in projection interneurons that synapse on MNs in the ventral horn but not in the MNs themselves. By analogy with En1/2 in ventral midbrain and Otx2 in the retina, we reasoned that En1 could be important for spinal MN survival. We used mice lacking one allele of En1 as a gain of toxic function model in vivo. Examination of the spinal cord of En1+/LacZ heterozygote mice revealed that similar number of small, medium and large α-MNs are present in En1+/LacZ mice and WT littermates at 1 and 3 months of age. At 4.5 months of age En1+/LacZ mice have about 20% fewer large α-MNs (>300µm2, VChAT positive) in the brachial and lumbar enlargements. The significant reduction in the number of α-MNs is associated with muscular weakness and reflex deficits. The loss of large α-MNs continues and at 15.5 months less than 50% of the large α-MNs remain in En1+/LacZ mice. There is no change in small and medium neurons, as in the human disease. As a first test of whether exogenous En1 can promote α-MN survival, we differentiated human iPSCs into spinal MNs. In our conditions virtually all differentiated cells are neurons (Tuj1 positive) and 80% are MNs (Islet1/2 positive) and after 5 days in vitro cells start to progressively die. If recombinant human En1 is added to the culture media, all MNs survive for at least 15 days in vitro. In addition to this pro-surviving activity En1 also stimulates the growth of large and complex neurites. Our results show that in vivo reduced En1 results in an adult onset loss of MNs that is progressive and accompanied by motor signs. In vitro En1 promotes the survival of differentiated iPSCs-derived MNs. We are currently testing hEn1 for in vivo α-MN survival activity.

**P95**

## Glial cell morphology, intracellular SOD1 distribution and elemental composition in the brainstem and hippocampus of the transgenic rat model of ALS

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Mutations in the Cu,Zn-superoxide dismutase (SOD1), which are the cause of 20% of familial ALS cases, increase the susceptibility of the SOD1 protein to form insoluble intracellular aggregations and cause copper and zinc unbinding which are necessary for normal monomer interactions and catalytic activity. This leads to a number of pathological intracellular changes including the inhibition of axonal transport, endoplasmatic reticulum stress, disruption of the proteasom system and mitochondrial dysfunction, all resulting in neurodegeneration and glial activation. The aim of this study was to investigate glial cell morphology, intracellular distribution of SOD1, and to perform complete in situ mapping of Cu and Zn and other physiologically relevant elements in the brainstem and hippocampus of the hSOD1G93A transgenic rat model of ALS. Immunohistochemistry for markers of astrocytes, microglia, neurons and SOD1 revealed a proliferation of glial cell, as well as progressive tissue accumulation of SOD1 in both the brainstem and hippocampus of ALS rats starting already at the presymptomatic stage, while neuronal degeneration was apparent only in the brainstem. Analysis of 3D confocal images of immunostained brain slices using the IMARIS software revealed a specific timeline of the glial cell response in the brainstem of ALS rats, with the activation of astrocytes coming first and before onset of disease followed by the activation of microglia. In the hippocampus astrocytes exhibited an identical reactive profile, while no changes in microglial cell morphology were observed. Additionally, ALS brainstem astrocytes demonstrated progressive SOD1 accumulation in the cell body and proximal cell processes, while microglial SOD1 levels were reduced and distributed mainly in distal cell processes. In the hippocampus both glial cell types exhibited SOD1 accumulation. X-ray fluorescence imaging revealed a decreased P and increased Ca, Cl, K, Ni, Cu and Zn in the brainstem tissue of symptomatic ALS rats, while the hippocampus of these animals contained higher levels of Cl, Ni and Cu, but lower levels of Zn. These results bring new insights into the timeline of the glial cells response during disease development and progression, and point to the disturbances in tissue elemental homeostasis as a prominent hallmark of disease pathology.

**P97**

## Development of a virally-induced TDP-43 in vivo model of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of upper motor neurons (MNs) in the brain and lower MNs in the spinal cord. Malfunctions of the nuclear protein transactive response DNA binding protein of 43 kDa (TDP-43) have been described in most ALS patients. Pathological TDP-43 mislocalizes to the cytoplasm, where it is hyper-phosphorylated, truncated and aggregated in inclusion bodies. However, the mechanism causing TDP-43 dysfunction and leading to neuronal death has not been identified yet. Recently, various transgenic TDP-43 rodent models have been generated either lacking or displaying fatal ALS pathology. Consequently, it has been challenging to investigate TDP-43 function or to use these models for drug screening studies, respectively. Thus, the aim of this project is to develop a novel TDP-43 mouse model showing specific and mild ALS pathology by injecting adeno-associated viruses (AAV) expressing human TDP-43 (hTDP-43) into neural regions comprising MNs. To this end, we injected hTDP-43 and/or GFP into the murine motor cortex, layer V, which has been described as one of the “starting points” of ALS in humans. Behavioral impairments will be monitored longitudinally for 6 months. Afterwards, cellular malfunctions caused by TDP-43 overexpression in the motor cortex will be investigated histologically and biochemically in these animals. First behavioral analyses revealed beginning of motor abnormalities 1 month after injection and significant deficits after 3 months. Together, we expect to generate an ALS model, which will mimic pathologies of early ALS and carries the potential to be used as tool for drug testing analyses.

P99

## Investigating the modifying role of EphA4 forward signaling in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects lower motor neurons in brainstem and spinal cord, and the upper motor neurons in the motor cortex, and leads to a progressive muscle phenotype in patients. ALS is characterized by considerable genetic and clinical heterogeneity, indicating that there are factors that modify the phenotypic expression of the disease. The tyrosine kinase receptor EphA4 was recently shown to be a modifier of ALS. Genetic and pharmacological inhibition of EphA4, a tyrosine kinase receptor, rescued the motor neuron phenotype in zebrafish and rodent models of ALS. In patients an inverse correlation was found between EphA4 expression and disease onset and survival. We further investigated the mechanism through which EphA4 affects motor neuron degeneration in ALS. EphA4 interacts with ephrin-A and ephrin-B ligands inducing signaling in the cell that bears the receptor (forward signaling) and the cell that bears the ligand (reverse signaling). Here, we aimed to determine the contribution of EphA4 forward and reverse signaling in ALS. To do so, we first reduced whole EphA4 receptor levels in the SOD1G93A mouse by crossing it to a mouse that constitutively lacks EphA4, generated by a gene-trapping method (EphA4.PLAP), a different approach from the one used in our previous work. We next reduced EphA4 forward signaling in the SOD1G93A ALS mouse model by crossing this mouse with two different knock-in mutant mice carrying either a substitution of the intracellular domain by eGFP or a single point mutation in the kinase domain of EphA4 (EphA4.eGFP and EphA4.KD). Although a reduction in whole EphA4 levels gave rise to enhanced survival, reduction of forward signaling did not significantly alter disease onset nor survival. To study whether a reduction in forward signaling increases axon sprouting, the percentage of innervated neuromuscular junctions in the gastrocnemius muscle was determined, but no differences were found. These results suggest that EphA4 signaling in ALS does not exclusively occur through forward signaling, and suggest a role for EphA4-mediated reverse signaling in neurodegeneration. In future experiments we intend to further explore the cellular mechanism of ephrin-mediated reverse signaling in the pathophysiology of motor neurodegeneration.

**P101**

## Phenotypic screening of PrP-hFUS-WT3 mouse model

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Mutations in Fused in Sarcoma (FUS), a nuclear DNA/RNA-binding protein, are causative for ALS. In order to validate a transgenic FUS mouse strain to model FUSopathy in vivo in ALS research, we have performed exhaustive phenotyping of a mouse model overexpressing human wild-type FUS. In this strain (The Jackson Laboratory stock 017916, developed by the group of Prof. C. Shaw), the expression of human FUS cDNA is driven by the mouse prion protein promoter (PrP) with N-terminal hemagglutin-tag (HA) in C57BL/6J background. Whereas hemizygous mice do not display neurological symptoms, homozygous mice develop progressive motor deficits and muscle atrophy starting as early as four weeks of age. At end stage, at nine weeks of age, severely paralyzed homozygous mice depict more than 50% motor neuron loss. To further evaluate the phenotype of the FUS mice, we screened the mice with an array of behavioural and biochemical tests. Although FUS protein levels are increased only around two-fold in the homozygous FUS mice, these mice have 14 copies of the human transgene as determined by digital droplet PCR. Motor performance on Rotarod and hanging wire tests disclosed rapid decline in strength and coordination between four and six weeks of age. Behavioural deterioration was accompanied by simultaneous dramatic drop in the amplitude of compound muscle action potentials (CMAP) at the level of the sciatic nerve, confirming the axonal loss during the disease course. Due to their reduced ability to gain weight, we screened the FUS mice for metabolic features. Unlike TDP-43 transgenic mice that suffer from gastrointestinal symptoms before the appearance of neuronal phenotype, FUS mice did not have abnormalities in mesenteric nNOS neurons. Measurements in calorimetric cages at early symptomatic age indicated that FUS mice are metabolically normal despite their small body size. At end stage, motor neuron loss in the ventral horn of lumbar spinal cord was accompanied by denervation of the gastrocnemius muscle and axonal loss at the sciatic nerve. Despite the inability of the FUS mice to gain weight, they did not seem to have metabolic or gastrointestinal problems. Motor neuron symptoms of FUS mice resemble closely those observed in mutant SOD1 mouse models.

However, in FUS mice motor defects and paralysis appear earlier and progress faster. In conclusion, FUS mice closely recapitulate an ALS phenotype and are thus a considerable option as an in vivo ALS model.

**PI03**

## Dual role of MHCI pathway in the development and progression of ALS in mouse models

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Increasing evidence suggest that the immune system plays an active role, both positive and negative, in the pathogenesis and progression of amyotrophic lateral sclerosis (ALS). However, the key participants that trigger and influence the immune response are not well defined. We previously showed that in motor neurons of transgenic SOD1G93A mice, the ALS mouse model that best mimics the human disease, there is a marked upregulation of the major histocompatibility complex I (MHCI) pathway both at the central and peripheral level. This phenomenon particularly occurs at the disease onset. Of note, we demonstrated that ALS mice with slow disease progression showed higher MHCI expression than the fast progressing mice and this was associated with a delayed muscle denervation, suggesting a role of MHCI in governing the disease course. To verify this hypothesis in the present study we examined the effect of removal of MHCI in transgenic SOD1G93A mice. As expected, the lack of MHCI in the peripheral nervous system (PNS) hampers the processes of axonal regeneration, anticipating the muscle atrophy and the disease onset. On the contrary, the lack of expression of MHCI, mostly by resident microglia in the spinal cord, positively influences the viability of motor neurons and the overall survival of SOD1G93A mice. This study provides, for the first time, a straightforward evidence for a differential role of immunity in the PNS versus the CNS in ALS animal models, highlighting the pivotal contribution of MHCI pathway and T cells in governing the progression of disease. These findings provide a possible explanation for the failure of immunomodulatory therapies that usually target both PNS and CNS and pave the way for new potential strategies to prevent the disease progression of ALS.
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**PI05**

## Molecular characterization of a TDP-43 loss of function endothelial phenotype

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Mutations in Tar DNA-binding protein of 43 kDa (TDP-43) cause the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 zebrafish loss-of-function mutants show endothelial hypersprouting and impaired directional migration thereby linking neurodegeneration to vascular dysfunction. The phenotype is evolutionary conserved in conditional endothelial TDP-43 knockout mice and TDP-43 deficient human umbilical vein endothelial cells (HUVEC). Known pathways to regulate sprouting angiogenesis are not affected. Instead, expression of the extracellular matrix protein fibronectin 1 (FN1), the vascular cell adhesion molecule 1 (VCAM1), as well as their receptor integrin α4β1 (ITGA4B1) is elevated. Importantly, partial knockdown (KD) of ITGA4, FN1, and VCAM1 homologues in the TDP-43 loss of function zebrafish rescues the angiogenic defects highlighting their physiological role in angiogenesis. FN1 RNA is a direct target of the RNA binding protein TDP-43 and is upregulated in motor neurons of ALS patients demonstrating the relevance of this TDP-43 target for human disease.

**PI07**

## TDP43 fragments clearance in a muscle model of sporadic ALS

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Amyotrophic lateral sclerosis is a fatal motor neuron (MN) disease that occurs mostly as sporadic. A common phenotype in affected MNs is accumulation of TDP43 in large inclusions in the cytoplasm. These inclusions usually contain fragments of TDP43: the most represented ones are the C-terminal fragments of 35 and 25 kDa. These fragments are hydrophobic and highly aggregation prone. The protein quality control (PQC) system is in charge of protein homeostasis. It relies on a chaperone network and on two main degradative systems: ubiquitin proteasome system (UPS) and autophagy. In this work we investigate which pathways are most involved in the degradation of this fragments using a muscle model of ALS. We transiently transfected C2C12 with plasmid coding for GFP-TDP43, GFP-TDP35 and GFP-TDP25. By immunofluorescence we observed that TDP43 was localized in the cytoplasm while TDP35 and TDP25 mislocalized in the cytoplasm forming large inclusions. We tried to detect this aggregates by filter retardation assay. Controversially, we found that TDP25 was less retained in FRA than TDP43. We speculate that this difference was related to the different degradation rate of the two different proteins. So we tried to isolate inclusions extracting protein sample in NP40 buffer. We found that TDP25 was mainly found in the NP40 insoluble fraction, confirming the need to use a stronger buffer to properly detect this aggregates. Subsequently we inhibited degradative systems and found that in muscle cells TDP25 aggregation was exacerbated when UPS was impaired. We tried to facilitate the UPS-routing of misfolded proteins overexpressing Bag1, a protein that acts in complex with Hsp70 in driving substrates to the UPS. We found that NP40 insoluble fraction of TDP25 was significantly decreased in presence of Bag1. It has already been demonstrated that UPS is impaired in ALS affected cells, so we wanted to test also a possible role for autophagy, even if it seemed poorly involved in TDP25 degradation at basal conditions. We overexpressed both HspB8 and Bag3. These proteins are part of the Hsp70 complex that leads substrates to autophagosomes internalization. We noted that both proteins exerted an anti-aggregation effect on TDP25 by both NP40 extraction and immunofluorescence. In conclusion we observed that also muscle cells are a site of TDP25 aggregation. We also found that tuning both degradative systems could exert a positive effect on the clearance of this fragment.

**PI09**

## Inwardly rectifying potassium channel Kir4.1 in microglial cell clusters in the hSODG93A rat model

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Previous research on ALS animal models showed that glial cells have a prominent role in motor neuron death and disease progression. One of the key roles of glial physiology is the maintenance of ionic homeostasis as a prerequisite for proper neuronal cell functioning. Potassium inwardly rectifying channel 4.1 (Kir4.1) has the major role in keeping potassium ion equilibrium in the central nervous system (CNS). Expression and role of Kir4.1 is studied in astrocytes and oligodendrocytes, while there is no indication of its expression in CNS microglia. Using immunofluorescence, we examined Kir4.1 expression in cervical and lumbar spinal cord of the hSODG93A ALS rat model. Although, general reduction in Kir4.1 expression in the gray matter of the end phase ALS animals was observed, islets of Kir4.1 demonstrating immunoreactivity similar to those shown in control animals was observed in the ventral horn gray matter. Utilizing microglial cell markers, Iba1, Cd11b, or Cd68 and the nuclear dye TO-PRO 3, Kir4.1-positive islets were identified as clusters of microglial cells. Kir4.1 and Iba1 positive clusters were also found in the ventral horn white matter of end phase ALS animals. Colocalization of signal intensity of immunofluorescence of microglial markers and Kir4.1 in the observed microglial accumulations as quantified by Pearson and Manders coefficients showed positive Kir4.1 and Iba1 signal colocalization, both in cervical and lumbar spinal cords of ALS rats, in contrast to control animals. Western blot experiments on primary microglial culture isolated from spinal cords of 2 days old transgenic hSODG93A rats and their non-transgenic littermates, showed increased expression of Kir4.1 in ALS animals compared to the control. These results for the first time demonstrate Kir4.1 expression in microglial cells in ALS which could indicate a K-channel – based change in physiology of reactive microglia as a specific pathological process.

**PIII**

## Expression of ALS-linked TDP-43 c-terminal domain reduces β-adrenergic-mediated cAMP signalling in cultured astrocytes

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TAR DNA-binding protein 43 (TDP-43) is a major component of pathological inclusions in motor neurons of patients with the adult-onset motor neuron disease amyotrophic lateral sclerosis (ALS). However, ALS inclusions are not restricted to neurons, but are found also in non-neuronal glial cells, in particular in astrocytes, which are in close contact with the synapses and contribute to motor neuron function. Cytoplasmic aggregation of TDP-43 in astrocytes alone is sufficient to cause motor neuron cell death in animal models, however the molecular mechanisms are poorly understood. Astrocytic and not neuronal adrenergic receptors are the main target of the noradrenergic system and the released noradrenaline, an essential neuromodulator in the central nervous system. By activating β-adrenergic receptors on the surface of astrocytes noradrenaline regulates many important processes, including astrocyte metabolic support to neurons, which may be altered in ALS. Whether pathogenic TDP-43 inclusion in astrocytes affect β-adrenergic mediated cAMP signalling and metabolism in astrocytes has not been studied yet. We expressed RFP-tagged wild type TDP-43 (TDP-43wt) and C-terminal fragment of TDP-43 (TDP-43208-414), which is involved in its cytoplasmic aggregation, in cultured astrocytes and monitored intracellular dynamics of cAMP signalling upon noradrenergic activation using real-time fluorescence microscopy and genetically encoded FRET-based cAMP biosensor Epac1-camps. In astrocytes expressing TDP-43208-414 red fluorescent inclusions typical for ALS pathology were observed in the cytosol of cells, while in TDP-43wt expressing astrocytes the signal was present mainly in the cell nuclei. Stimulation of cells with noradrenaline induced an increase in the FRET ratio signal, in either type of astrocytes, reflecting the intracellular cAMP increase due to activation of β-adrenergic receptors. However, the amplitude of cAMP signalling was significantly reduced in TDP-43208-414 expressing astrocytes compared to TDP-43wt expressing astrocytes, as reflected by lower maximal FRET increase (20% vs. 27%, respectively; $P<0.05$). We have observed a reduced number of β2-adrenergic receptors in TDP-43208-414 compared to TDP-43wt expressing astrocytes.

The observed downregulation of β2-adrenergic receptors and the consequentially reduced cAMP signalling may affect downstream metabolic processes in astrocytes in the presence of the ALS linked TDP-43208-414 inclusions.

PII3

## Excitability and calcium homeostasis of mutant SOD1-D90A iPSC-derived motor neurons

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Point mutations in the gene coding for superoxide dismutase 1 (SOD1) are responsible for approximately 20% of familial amyotrophic lateral sclerosis (ALS) cases. The mutated Cu/Zn-SOD1-protein acquires a toxic gain-of-function, accumulates in spinal cord motor neurons and is responsible for the degeneration of these cells. While the mode of inheritance of mutant-SOD1-associated ALS is typically autosomal-dominant, both recessive and dominant patterns of inheritance have been described for aspartate-to-alanine mutations in codon 90 (D90A) (Al-Chalabi et al., 1998). For better understanding of the mode of action of mutant SOD1 in the development of the disease and mechanisms underlying impairment of motor neuron function, we have used the CRISPR/Cas9-technologyfor homozygous and heterozygous correction of the D90A-SOD1 point mutation in induced pluripotent stem cells (iPSC) derived from patient fibroblasts. So far, both the corrected homozygous cell line and the isogenic patient cell line have been differentiated into motor neurons using an already established protocol. Motor neurons were examined by calcium imaging in order to reveal possible differences in calcium homeostasis and ligand (glutamic acid, GABA, acetylcholine and glycine) activated ion channel function. iPSC-derived motor neurons will additionally be examined by the patch-clamp technique to examine mutation-specific electrophysiological properties. Via identification of differences between the fully corrected and the homozygous and heterozygous patient cell lines we aim to characterize a potential dose dependent impact of the D90A-SOD1 mutation on motor neuron excitability and calcium homeostasis.

**PII5**

## Gene profiling of human iPSC-derived motor neurons from sporadic ALS patients reveals a participation of mitochondria in the autonomous mechanisms

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Background: Because the most prevalent sporadic ALS form is not genetically inherited, attempts to translate therapeutic strategies have failed because the described mechanisms of disease are based on animal models carrying specific gene mutations and thus do not address sporadic ALS.
Methods: In order to achieve a better approach to study the human disease, human induced pluripotent stem cell (hiPSC)-differentiated motor neurons were obtained from motor nerve fibroblasts of sporadic ALS and non-ALS subjects using the STEMCCA Cre-Excisable Constitutive Polycistronic Lentivirus system and submitted to microarray analyses using a whole human genome platform. REVIGO and DAVID analyses of differentially expressed genes identified molecular function and biological process-related genes through Gene Ontology. The String was used to predict protein interactions, based on the confidence between two nodes.
Results: REVIGO highlighted the related functions mRNA and DNA binding, GTP binding, transcription (co)-repressor activity, lipoprotein receptor binding, synapse organization, intracellular transport, mitotic cell cycle and cell death. KEGG analyses showed pathways associated with Parkinson's disease and oxidative phosphorylation, highlighting iron homeostasis, neurotrophic functions, endosomal trafficking and ERK signaling. The DAVID analysis focused on the Cellular Component Ontology (CCO) of differentially expressed genes and has pointed to 23 enriched GO terms under high stringency conditions. The CCO indicated four GO terms related to mitochondrion in hiPSC derived motor neurons. Cellular component terms related to mitochondrion gene list were, then, organized and submitted to STRING. This analysis generated 105 nodes and 232 edges based on the confidence score.
Conclusions: Gene profiling of differentiated motor neurons from sporadic ALS patients indicates mitochondrial dysfunction as a key factor in this cell-autonomous neurodegeneration process.
Funding: This work was supported by FAPESP and CNPq, Brazil.

**PII7**

## A novel human in vitro model of motor neuron disease (MND) uncovers individual patient response to antioxidant drugs

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Background: Mechanisms leading to progressive loss of motor neurons (MNs) in MND remain unclear. Oxidative stress is a major cellular insult implicated in multiple neurodegenerative diseases, including MND. It contributes to glial pathology through damaging cellular DNA/RNA, thus triggering DNA damage response. Keap1-NRF2 pathway, which regulates cytoprotective response to oxidative stress, is an important target for antioxidant drugs in neurodegeneration. Using a cell reprogramming method to convert human fibroblasts from living MND patients to induced neuronal progenitor cells (iNPCs) and subsequent differentiation into astrocytes (iAstrocytes), we have established an iAstrocyte-MN co-culture system that reproduces astrocyte toxicity against MNs. This tool allows studying MND while patients are still alive and can potentially help advance personalised medicine.
Aims of the study: 1. To assess the presence of oxidative stress in human biosamples by quantifying 8-OHdG in CSF samples of MND patients versus controls and post-mortem CNS; 2. To assess the extent of oxidative damage in familial and sporadic patient iAstrocytes versus controls; 3. To perform a drug screening testing chemical compounds which reduce oxidative stress.
Results: Levels of oxidative damage and oxidised RNA in MND patient CSF samples are significantly higher than in controls. Immunohistochemistry of 8-OHdG also showed high levels of reactivity in cortical neurons and spinal cord MNs of MND patients, reflecting increased oxidation levels in this group when compared to controls. Consistently, level of cellular oxidation in MND patient-derived iAstrocytes is significantly higher than in controls. A significant rescue of MNs was observed in the drug screening system using antioxidant compounds. Interestingly, patients carrying different mutations seem to respond to different antioxidant drugs.
Conclusion: We show that iAstrocytes reproduce aspects of MND pathology in vitro and reflect different levels of oxidative stress seen in human post-mortem tissues and CSF. Overall, oxidative stress levels in patient biosamples and derived cells are significantly higher when compared to controls. Remarkably, co-cultures demonstrated that MND iAstrocytes toxicity can be dampened by using antioxidant drugs and patients carrying different mutations respond to different drugs. These data indicate that our co-culture system has the potential to help advance personalised medicine approaches.

**PII9**

## NF-kB constitutively activated in astrocytes enhances microglia response and induces a biphasic effect on MNs performance during ALS disease course

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Glia-mediated neuroinflammation is thought to play a role in the pathogenesis of ALS; whereas the impact of microglia activation has been shown to be detrimental and the effect of astrocytes is still unclear. In particular, suppression of NF-kB module in astrocytes has produced no obvious impact in the SOD1(G93A) mouse model.
In our study we use a genetic strategy based on astrocyte-specific expression of a constitutively active IKK allele under doxycycline control and we show that astrocyte modulate strongly microglial responses and may have significant beneficial or detrimental effects depending on the disease stage. The activation of IKK/NF-κB in astrocytes from P20 results in a massive expansion of microglia (up to 8-fold) due to prominent proliferation, blood spinal cord barrier breakage and leukocytes infiltration. However, pre-symptomatic phase is significantly prolonged in these mice, whereas progression phase is shortened, resulting in unchanged overall survival compared to single/tg SOD1 littermates. Misfolded SOD burden and autophagy markers are improved by IKK/NF-kB activation in the early stages (until P70) but not in later stages. Microglia polarization profiles markedly change over time: the expanded microglia was predominantly M2 polarized in early stages but shifted to M1 polarization after P80. We employ the DOX control on the IKK/NF-kB activation to show that the beneficial and detrimental phases can be dissociated; restricting IKK-CA expression to the pre-symptomatic phase (before P80) significantly prolongs overall survival, whereas activating IKK-CA only in the progression phase (from P80) shortens overall survival. Microglial proliferation is comparable in the continuously-activated and in late-activated mice, suggesting that astrocytes modulate proliferation but not the polarization of microglia. We explored the factors involved in astrocyte-controlled microglia expansion, identifying Wnt5a as a candidate mediators; the blockade of Wnt secretion was sufficient to suppress IKK/NF-kB-induced microglial proliferation and to reverse the beneficial effects on MN disease markers observed in the early phase. Our findings suggest that boosting early-stage protective microglial response is a possible intervention for the treatment of presymptomatic gene-carrier patients.

**PI21**

## Biomarkers of inflammation in long-term G-CSF treated ALS patients

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Objectives: Assessment of disease and intervention related changes in neuroinflammation in ALS patients who were treated with G-CSF (filgrastim) over a long time.
Introduction: G-CSF is a well-known and safe hematopoietic growth factor that potentially compensates neuronal loss in ALS patients by neuroprotection, neurogenesis and immunomodulation. Inflammation, which is considered a major contributor to neurodegeneration, may be attenuated by G-CSF.
Patients and Methods: We treated 37 ALS patients (26m, mean age at start 52.1 yrs., mean ALSFRS-r at start 38.5) with G-CSF in addition to standard therapy after informed consent and on a named patient basis. Application modes and doses were individually adapted (range 6-816, mean 351 Mio. IU/month s.c.). Patients were seen monthly, we obtained ALSFRS-r, clinical chemistry, as well as immune parameters with electro-chemiluminescence, blood smears and bone marrow mobilization parameters throughout the long-term intervention of up to over 5 yrs.
Results: We found safety and compliance to be excellent. G-CSF was well tolerated and resulted in effective hematopoietic stem cell mobilization. We demonstrated an immediate effect of G-CSF application upon different cytokines mediating systemic inflammation; this was also present over time. Patterns of pro-and anti-inflammatory cytokines prior to first G-CSF treatment were significantly different in patients with higher vs. lower survival. G-CSF modulated many of these cytokines over time. Further, increased G-CSF induced hematopoietic stem cell mobilization capacity was associated with higher survival in our patients.
Conclusion: Cytokines represent promising biomarkers predictive of clinical course and possibly also treatment effects. Long-term administration of G-CSF in ALS patients is safe and well tolerated. G-CSF treatment leads to persistent changes in pro- and anti-inflammatory cytokines and hematopoietic stem cell mobilization in ALS patients, and thus is a promising therapeutic candidate in ALS.

**PI23**

## Dysregulation of ROCK and ERK in SOD1G93A mice: combinatorial ROCK/ERK-inhibition as possible therapeutic approach?

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The Rho-kinase (ROCK) and the Extracellular-signal regulated kinase (ERK) and their downstream targets are important modulators of the actin cytoskeleton, which has been shown to be affected in amyotrophic lateral sclerosis (ALS). Dysregulations of both pathways have been described in ALS before. A bilateral ROCK-ERK information flow has been identified in healthy neurons, which becomes shifted towards a unidirectional crosstalk in a cellular model of spinal muscular atrophy (SMA). We have discovered dysregulation of both pathways in spinal cord tissue of SOD1G93A mice and of sporadic ALS patients. Beneficial effects of ROCK inhibition have already been demonstrated in SOD1G93A mice before. Based on recent discovery of motor neuron disease-related changes in the crosstalk of the two pathways, our strategy is now to administer a combinatorial ROCK-ERK inhibitory treatment in SOD1G93A mice. Animals are treated with fasudil and selumetinib daily by intraperitoneal injection, beginning at 70 days of age or permanent via food uptake, beginning at 60 days of age. Mice are monitored by behavioural tests (rotarod, footprint and hanging-wire) weekly and scored daily for general condition. Nerve conduction and motor unit number estimation studies are performed during the symptomatic stage. We could demonstrate a pathophysiological role of ROCK/ERK pathways in SOD1G93A mice and human post mortem tissue on protein and mRNA level, but now we could not show positive effects of our combinatorial ROCK/ERK inhibitory treatment. Rather we could detect a worsening tendency in motor function, general condition and especially in survival. We are currently analysing whether there is a negative influence of selumetinib treatment on the previously reported beneficial effect of fasudil in SOD1G93A mice.

**PI25**

## An assessment of treatment guidelines, clinical practices, demographics, and progression of disease among individuals with ALS

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Background: There continues to be a need for new therapies to treat amyotrophic lateral sclerosis (ALS). Edaravone (MCI-186) has been investigated in Japanese patients with ALS using the Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised (ALSFRS-R).
Objectives: Assess clinical practice and treatment guidelines, and compare the progression of disease among patients with ALS in Japan, Europe, and the US.
Methods: To assess similarities and differences between Japanese, European, and US medical practice for ALS, we reviewed country-specific practice guidelines. We also performed a literature review to compare the demographics and baseline characteristics of ALS for Japanese, European, and US populations. Using reference studies of ALS in the US and Europe, and edaravone studies in Japan, progression of ALS disease was assessed in patients receiving placebo using a random coefficient model, with an assumption that ALSFRS-R score declines in a linear fashion within each patient. The changes per month in ALSFRS-R score were calculated and compared between the studies, and with published longitudinal data from the Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) database.
Results: Overall, diagnostic criteria, recognition of ALS symptoms, comorbidities, use of riluzole as the first-line therapy, nutrition, and respiratory support were similar across guidelines. There were no clear differences in the incidence of sporadic ALS (range, 91-98%) and bulbar onset (range, 11-41%), time from onset to diagnosis (range, 9-14 months), and use of percutaneous endoscopic gastrostomy (range, 8-58%) among the Japan, EU, and US populations. However, use of tracheostomy-based invasive respiratory support was higher in Japan (29–38%) than in the EU (1–31%) or the US (4%). Progression of disease, as assessed by ALSFRS-R score in patients receiving placebo, was similar between the reference studies including the US and EU population (range across 10 studies, -0.89 to -1.60 points/month) and edaravone studies in the Japanese population (range across 2 studies, -1.03 to -1.21 points/month). These results parallel with published data from the PRO-ACT database (-1.02 points/month).
Conclusion: Clinical practice and treatment guidelines for ALS regions are similar with the exception of tracheostomy use. Disease progression rates do not appear to differ. Japanese ALS clinical trial experience may be generalizable to US and EU ALS patients.

**PI27**

## Human mesenchymal stem cell therapy reduces neuroinflamation in earlier symptomatic stages of ALS in SOD1-G93A mice

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Amyotrophic lateral sclerosis (ALS) is a human fatal neurodegenerative disease characterized by progressive loss of upper and lower motoneurons. To date, the pathogenic mechanisms responsible for triggering the neurotoxicity and death of motoneurons is poorly understood, although inflammation and oxidative stress are critically involved. Cell therapy is emerging as a promising approach for the treatment of such neurodegenerative disease. In this sense, human adult mesenchymal stem cells (hMSC) derived from adipose tissue can be easily obtained by lipoaspiration. Furthermore, we have previously reported that xenografted hMSC are neuroprotective and lead to immunomodulation following spinal cord ventral root avulsion. Thus, we investigated the role of hMSC therapy in the earlier symptomatic stages of ALS in G93A mice, a well-established ALS model. For that, hMSC derived from adipose tissue were administered in 70 days old animals (pre-symptomatic stage). A single injection of 1x105 cells was carried out via tail vein in anesthetized mice. The animals were sacrificed at early symptomatic stage (100 days old), and the lumbar spinal cord was dissected out and processed for immunohistochemistry (anti-Iba1 – microglial marker) and for qRT-PCR to evaluate the pro-inflammatory cytokines. The results showed that treatment with hMSC reduced the microglial activation by 30% when the density of pixels was evaluated in the ventral horn of the lumbar spinal cord. Also, significant improvement in motoneuron morphology could be observed. Furthermore, we show that hMSC treatment downregulated 2 to 3-fold the pro-inflammatory cytokines IL1β and TNFα when compared to the vehicle group ($p>0,001$). Altogether, our results demonstrate that hMSC can be a potential therapy for ALS by immunomodulating neuroinflammation, and possibly by providing neurotrophic substances nearby the affected motoneurons.

P2

## Vascular basement membrane proteins in plasma of ALS patients are associated with degree of cortical perfusion and age at sporadic disease onset

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Factors leading to development of sporadic amyotrophic lateral sclerosis (ALS) are poorly understood. It is believed, that clinical disease symptoms are preceded by a long pre-clinical phase where numerous unknown factors can contribute to the timing of onset ranging from 20 to 80 years of age. Since ALS patients show evidence of decreased cerebral blood flow dynamics, our aim is to explain the mechanisms of vascular dysfunction in ALS etiology. We found that spinal cord blood vessels in SOD1(G93A) ALS mice show separation of vascular and astrocyte-derived basement membrane and perivascular astrocytes detach from vessels. We found that this form of vascular dysfunction results in transcriptional activation of basement membrane gene ontology group in human sALS (FDR p=0.003) and SOD1 mouse spinal cords at onset (p=0.0003) and pre-onset stage (p=0.003). Using immune bead proteomics platform we found that increase of laminin and collagen basement membrane proteins in blood plasma of ALS patients (n=19) is significantly correlated with the decrease of cerebral 18 FDG perfusion in the temporal lobe (laminin r = -0.51, collagen r = -0.52) and younger age at sporadic disease onset (r = - 0.46 and -0.52). In summary our findings point that vascular dysfunction can be an important factor contributing to changes in cerebral blood flow levels and is one of the factors associated with the timing of ALS onset. Monitoring of basement membrane proteins in plasma can become a novel biomarker of vascular dysfunction in ALS.

**P4**

## Analysis of chitotriosidase activity in plasma and CSF as a candidate biomarker for amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron degenerative disorder that is lack of clinical biomarkers. The contribution of inflammation to ALS is increasingly recognized, although it is yet not well understood. It is known that degeneration is accompanied by inflammatory responses with microglia activation. Human chitotriosidase (ChT) is an enzyme selectively expressed in chronically activated tissue macrophages, which has demonstrated an important role in inflammation in storage systemic diseases. ChT also seems to play a role in neuroinflammation as it has been found to be increased in several neurodegenerative diseases.
Objective: The aim of this study was to measure ChT activity in CSF and plasma in 32 patients versus 46 control subjects.
Materials and methods: Patients with ALS diagnosis (n=32) and controls (n=46) were recruited in our hospital. Patients were classified as bulbar or spinal ALS and several variables were recorded including ALSFRS-R scale, onset of mechanical ventilation, PEG, Riluzole treatment, cognitive impairment and serum CK levels. Plasma and CSF ChT activity was measured using the fluorogenic substrate 4MU-chitotrioside. Genotyping for the 24-bp insertion in exon 10 of the CHIT1 gene was performed. Statistical analysis was performed with SPSS 21.0 (IBM, Inc., USA). ChT activity data represents median (range), as it does not follow a normal distribution. Mann-Whitney test was used to assess differences in ChT activity between patients and controls. The study was approved by the local Ethics Committee.

Results: Plasma ChT activity was similar between ALS patients [65.01 (42.34)] and controls [71.52 (41.03)] ($p=0.943$). On the contrary, CSF ChT activity was significantly increased in ALS patients [11.43 (89.35)] compared to controls [3.03 (2.42)] ($p<0.001$). In a logistic regression model adjusted for age and gender, CSF ChT activity was an independent predictor of disease status (ALS vs control) ($p=0.001$).
Conclusion: This study shows that ChT activity is significantly higher in CSF of ALS patients than in control subjects, becoming a candidate biomarker. We postulate that the increase of CSF ChT activity reflects a sign of neuroinflammation, supporting the role of inflammation as a pathogenic pathway of disease development. Therefore, neuroinflammation represents an important therapeutic target.

**P6**

## Lipidomic signatures in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis is the most common adult-onset motor neuron disease characterized by the degeneration of upper and lower motor neurons in the brain and spinal cord and by a survival of only 2-5 years after symptom onset. We performed an untargeted lipidomic approach in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis (n=40) compared to a set (n=45) of other neurological diseases. Lipidomic analysis was performed with liquid chromatography coupled with high resolution mass spectrometry. LipidSearch and TraceFinder softwares were used to characterize lipid species. One hundred and twenty-two lipids were considered the most robust among the 200 detected and were subjected to a stringent statistical modelling combining univariate and multivariate analyses with the biosigner algorithm that use itself a combination of machine learning approaches. Compared to other neurological diseases, amyotrophic lateral sclerosis patients display a specific and highly significant lipidomic signature in their CSF, using the different statistical tools, involving phosphatidylcholines, plasmalogens, lysophosphatidylcholines, glucoceramides, sphigomyelins and triglycerides with long chain fatty acids, most of them being increased in amyotrophic lateral sclerosis. The phosphatidylcholine PC(36:4) was the strongest biomarker of the diseases found by all statistical tools. This signature partially overlapped those found in the brain of amyotrophic lateral sclerosis mice model with superoxide dismutase 1 disruption, with a common increase of several phosphatidylcholines. Significant predictions of clinical evolution and survival were also evidenced with a strong contribution to the profile of triglycerides with long chain fatty acids. Our study evidenced a large remodeling of lipids in the cerebrospinal fluid of amyotrophic lateral sclerosis patients with specific signatures of the disease and new biomarkers of evolution.

**P8**

## TDP-43 function in nervous tissue is essential for physiological autophagy

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Introduction: The cause of the ALS in most cases is unknown, yet several familial forms shed light upon its pathogenesis. Invoked disease mechanisms include cell stress, defective autophagy, bioenergetics failure, abnormal proteostasis, to name a few (1,2). TDP-43 is a nuclear RNA binding protein that is aggregated and misslocalized in the cytosol in 97% of spinal cord from many non-familial ALS ALS patients, irrespectively of bulbar or non-bulbar onset. TDP-43 has several functions regulating RNA metabolism. One of these functions is the inhibition of non-conserved (cryptic) exon splicing (4). The aim of this work is to analyze TDP-43 repression splicing function as a potential biomarker for ALS and as a potential disease mechanism.
Materials and Methods: ATG4B and GPSM2 genes present cryptic exons normally spliced by TDP-43. We setup a method for evaluating cryptic exons abundances from lumbar spinal cord (LSC), motor cortex (MC), occipital cortex (OC) and brainstem (BS) from pathology samples as well as in vitro cell cultures by using SYBR Green based RT-qPCR. HeLa cells were used for Western Blot and Immunofluorescence analyses.
Results and Conclusions: The abundances of ATG4B and GPSM2 cryptic exons are increased significantly in LSC, MC, OC and BS samples in all analyzed ALS patients, suggesting a central nervous system widespread loss of function of TDP-43. Furthermore, there were significant differences between regions and disease onset, confirming a different impact of the disease in the analyzed tissues and phenotypes. The high values on ROC(<0.8) curves indicate a potential use of cryptic exon abundances as sporadic ALS biomarker. Further, a TDP-43 knockdown cell model revealed an important role of this protein in autophagy, leading to loss of ATG4B function non compensated by other ATG4 isoforms, with increased p62-SQTM, in common with findings of selected ALS cases. In conclusion, this novel method can be useful as a biomarker and drug discovery focused on TDP-43 function, and reveals the potential importance of TDP-43-autophagy axis in the pathogenesis of ALS.
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**P10**

## Autoimmunity profiling of amyotrophic lateral sclerosis plasma

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Amyotrophic lateral sclerosis (ALS) is the most common neurodegenerative disease in adults that results in muscular paralysis, followed by death within 3-5 years after onset. The pathogenesis and aetiology of ALS is not known, which explains why there is still no cure and the efficiency of the existing treatments is low. Genetic factors have been found to influence rate of progression, neuronal degeneration and increase susceptibility to the disease, while most autoimmune mechanisms remain somewhat unexplored. Other neurological disorders, such as Multiple Sclerosis, have known autoimmune components and the relevance of investigating these in ALS is becoming larger. Accumulative evidence from biochemical-, morphological-, pharmacological- and physiological studies suggest the existence of such components. However, evidence from larger proteomic studies is lacking. By using a proteomic approach, the aim of our study is to investigate the occurrence of auto-antibodies in human plasma samples from ALS patients. Plasma samples from 233 ALS patents and 204 healthy controls were profiled in this study. Initially, an untargeted screening on a high density planar protein array, containing 42.000 protein fragments representing over 19.000 proteins, was done using a small sample pool. This was followed by a targeted multiplexed bead based suspension array, with 384 protein fragments immobilized on magnetic beads. Detection and profiling of antibody expression levels in all 437 samples enabled identification of differences in antibody expression between ALS and control samples. Additionally, antibody expression levels in frontotemporal dementia (FTD) were included in the comparison. Preliminary data from the untargeted screening revealed that ALS patients have auto-antibodies towards several protein fragments, including EPHA3 in the ephrin receptor subfamily of the protein-tyrosine kinase family, which have shown association to ALS in previous studies. With this study, we wish to increase the insight and knowledge of this unexplored area, and hopefully contribute to further understanding the pathology and aetiology of ALS.

**PI2**

## Small fiber neuropathy characterization in the SOD1G93A ALS mouse model

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In the last years, cumulative data have shown that patients with amyotrophic lateral sclerosis (ALS) and mouse models of the disease present loss of small epidermal and dermal nerve fibers and sensory dysfunctions, in addition to the classical motor symptoms. Our objective is to characterize this small fiber neuropathy and to clarify if axonal loss involves all sort of fibers equally, or if there is some specificity. For this purpose, we performed an immunohistochemical characterization of total intraepidermal nerve endings (protein gene product; PGP9.5), peptidergic (calcitonin gene-related peptide; CGRP) and nonpeptidergical nerve epidermal endings (isolectin B4; IB4) of the SOD1G93A mouse at different stages: presymptomatic stage (8 weeks), disease onset (12 weeks) and in symptomatic stage (16 weeks). The sympathetic sweat gland innervation was immunolabeled for vasoactive intestinal peptide (VIP) from very early stage (4 weeks) to disease onset (12 weeks). The results showed a marked reduction of the intraepidermal nerve fibers already in the presymptomatic stage compared to the wildtype littermates ($p<0.05$). This axonal loss affected more markedly the nonpeptidergic axons from the disease onset stage (39% axonal loss, $p<0.05$), whereas no significant differences were found in the CGRP positive fibers (14.3% axonal loss). A reduction of the sympathetic innervation of the sweat glands was also found from the disease onset stage (29% axonal loss, $p<0.05$). In summary, we have found that nonpeptidergic and sympathetic innervation of the skin are predominantly affected in the SOD1G93A mouse model. These findings suggest that this specificity could be used as an accessible biomarker for the disease.

**PI4**

## Protein array enabled profiling of autoantibody repertoires in ALS, ALS-FTD and FTD

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The general understanding of the global reactivity patterns in the human autoantibody repertoires are still at an early phase. More and more diseases and conditions are speculated to have autoimmune components but very few novel targets are clearly associated to disease conditions. In order to explore autoantidbody reactivities and to screen for novel autoantigens, we have for several years produced and utilized various formats of protein fragment arrays with antigens from the Human Protein Atlas. Through a combination of various planar and bead-based microarray formats, including an array with 42.000 protein fragments representing 19.055 unique proteins, assays are set up both for broad screening studies as well as targeted analysis for verification and validation of initial findings. The latter format utilize a format where 384 samples can be analyzed in parallel on 384 antigens. We see in general a very large degree of heterogeneity between individuals and also often relatively high numbers of antigens targeted by each individuals repertoires of IgGs, which is also the case for healthy individuals. Within a larger effort of autoantibody profiling in large numbe of CSF and plasma samples in a broad neurodegenerative and psychiatric disorder context, have we here focused on a comparison between the autoantibody repertoires in ALS, ALS-FTD and FTD.

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**P16**

## Low IDL-B and high LDL-1 subfraction levels in serum of ALS patients

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Introduction: Converging evidence highlights that lipid metabolism plays a key role in ALS pathophysiology. Dyslipidemia has been described in ALS patients and may be protective but peripheral lipoprotein subclasses have never been studied. Such knowledge may improve our understanding of these pathways in ALS.
Materials and methods: We collected sera from 30 ALS patients and 30 gender and age-matched controls. We analyzed 11 distinct lipoprotein subclasses including very low–density lipoproteins (VLDLs), three intermediate-density lipoproteins (IDL A, IDL B, and IDL C), seven LDLs (1-2: large LDLs, 3-7: small LDLs), and High Density Lipoprotein (HDL) by linear polyacrylamide gel electrophoresis (Lipoprint, Quantimetrix Corporation, USA). We also measured lipoprotein(a), apolipoprotein B, and apolipoprotein E levels. We compared the lipoprotein profiles between both populations and evaluated their link to clinical status and evolution.
Results: ALS patients had significant higher total cholesterol, HDL-cholesterol, and LDL-cholesterol levels than controls ($p < 0.0001$, $p = 0.0007$, and $p = 0.0065$, respectively). The LDL-1 subfraction concentration was higher (27.26 ± 10.65 mmol/L vs 1.029 ± 0.406 mmol/L; $p = 0.0006$) and the IDL-B subfraction lower (8.0 ± 2% vs. 6.5 ± 2%; $p = 0.001$) in ALS patients than controls. There was no difference in all apolipoprotein concentrations between the two groups. We observed no association between these parameters and the main clinical parameters.
Discussion: Our results confirm the association between ALS and dyslipidemia. The low IDL-B levels may explain the hepatic steatosis frequently reported in ALS, due to an increase in hepatic uptake of these particles. The high levels of the cholesterol-rich LDL-1 subfraction is consistent with previously reported hypercholesterolemia.
Conclusion: This study describes, for the first time, the distribution of serum lipoproteins in ALS patients, with low IDL-B and high LDL-1 subfraction levels. These results open new prospects to understand more precisely lipid metabolism alterations

**PI8**

## Expression of carnitine/acylcarnitine translocase in patients with motor neuron disease

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Introduction: The cholesterol metabolism and mitochondrial homeostasis have been implicated in the pathogenesis of neuronal loss in motor neuron disease (MND). SLC25A20 encodes the carnitine-acylcarnitine translocase, which transports fatty acids into the inner mitochondrial membrane for β-oxidation process.
Aims: The aim of the study was to analyze the expression of SLC25A20 in patients with MND.
Material and methods: The study was performed in PBMCs of 63 MND patients (38 with classic ALS, 10 with PBP, and 15 with PMA) and 48 healthy age- and gender-matched controls. The mean patients' age was 58.0 ± 12.5 years, and the diagnosis delay 14.45 ± 17.9 months. The mRNA expression of SLC25A20 was studied by real-time qPCR and, while the protein level in blood serum by Western blotting.
Results: 33 patients presented with hypecholesterolemia, while 25 patients with increased LDL level. The expression of SLC25A20-mRNA was significantly higher in MND patients compared to the control group ($p<0.0001$), and it was independent of cholesterol level in blood serum. SLC25A20 expression was higher in patients at all ages and with every clinical phenotype, when compared to the matched controls ($p<0.05$ and $p<0.01$, respectively). In patients with increased LDL level, the expression of SLC25A20 was similar to the control group but in patients with normal LDL level, it was significantly higher ($p<0.0001$). The results obtained by real-time qPCR were confirmed on the protein level.
Conclusion: Patients with MND and high level of LDL may not need to compensate energy deficiency by higher expression of carnitine/acylcarnitine translocase.

**P20**

## Multidisciplinary care improves survival of patients with ALS – evidence from the Ljubljana ALS Centre

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Background: Some research has been done on comparison of survival of patients with ALS attending multidisciplinary ALS clinics and those attending general neurological clinics (1,2). However, the current literature lacks data on the effect of multidisciplinary approaches on survival within the same ALS centre over the years. In 2003, the Ljubljana ALS Centre was founded at the University Medical Centre Ljubljana. The aim of our study was to determine whether clinical features and survival of patients with ALS have changed since the early beginnings of our multidisciplinary centre.
Methods: 124 patients were included in our retrospective cohort study. From the time of enrolment in our ALS centre, every patient was followed for a period of four years. Comparison was made between the patients diagnosed with ALS in years 2003-2005 (early group) and those diagnosed in years 2011-2012 (late group). Chi square test was used for comparison of the clinical features between the two groups. Kaplan-Meier survival analysis was performed to compare survival.
Results: In comparison to the early group, there was a significant increase in the use of non-invasive ventilation and riluzole treatment in the late group (chi square, $p=0.001$ and $p<0.001$, respectively). Kaplan-Meier survival analysis showed a significant improvement of survival in the late group compared to the early group (log rank, $p=0.005$).
Conclusions: Our findings suggest that the multidisciplinary approach at the Ljubljana ALS Centre has improved the survival in patients with ALS in the last ten years. Further research is needed to identify the potential independent factors of prolonged survival in our patients.
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P22

## The phenotypical variability of ALS and its relationship with survival. A 25 year experience in Catalonia

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Introduction and objectives: ALS is recognized as a progressive neurodegenerative disorder with a mean survival of 3-5 years after diagnosis. However, it is increasingly recognized that there is a huge heterogeneity within the disease, with genetic, nutritional and epidemiological factors that can modify prognosis. The phenotype at onset is a well documented prognostic marker.
Methods: Analysis of a historical cohort of patients referred to the ALS unit at Hospital Universitari de Bellvitge in Barcelona, Spain (1986-2013).
Results: We reviewed a total of 282 ALS patients. Spinal phenotype accounted for the majority of patients with 144 cases (51,1%) not including 9 respiratory onset patients (3.2%). They were followed by bulbar onset patients with 69 cases (24.5%). Atypical phenotypes accounted for 60 cases (21.3%) and were distributed by frequency as follows: 19 patients met criteria for PMA (6.7%), 10 PLS (3.5%), 11 PBP (3.9%), 9 flail leg (3.2%), 5 flail arm (1.8%), 2 hemiplegic forms (Mills' syndrome), and 2 pure monomielic forms (0.7%). Mean survival time was 38 months (CI 33,5 – 42,5) for spinal patients, and 33 months (CI 30 - 35) for bulbar patients. All atypical phenotypes were associated with increased survival times. The most favorable outcome was for PLS patients with a mean survival of 183 months (CI 53 - 312) acknowledging some variability. For PMA it was 71 months (48.2 – 93.8), PBP had a clearly better outcome than bulbar onset ALS with 46 months (CI 25.5 – 66.5). While flail leg syndrome seemed to have a slightly better outcome than PMA with 86 months survival (CI 27 – 144), the rate of progression is variable. Of our 5 phenotypically confirmed flail arm patients, only one died 2 years into his disease with the remaining 4 alive at months 81, 102, 148 and 157.
Conclusions: The phenotype at onset is a solid prognostic marker in ALS patients. Atypical forms are associated with better outcomes in terms of survival and should be taken into consideration both for individual prognosis and for the design of clinical trials.

**P24**

## Infrastructure and resources for ALS research

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Prize4Life (P4L) is a non-profit organization dedicated to accelerating the development of effective treatments and cure for ALS. P4L was founded and managed by ALS patients who assumed responsibility for fighting the disease and affecting the destiny of future patients. P4L works by creating infrastructure to support ALS researchers, facilitate and advance research efforts and encourage collaboration. The Pooled Resource Open-Access Clinical Trial database (PRO-ACT): The largest-ever ALS clinical database (www.alsdatabase.org), containing data of over 10,000 ALS patients collected during 23 ALS clinical trials conducted over the past 25 years. Data was de-identified, standardized and harmonized to create a unified, easy-to-use database. The database includes longitudinally sampled data, providing rich and detailed information of the progression, antecedents and consequences of ALS. PRO-ACT is an open-access database freely available to researchers from academia and industry worldwide. The ALS Research Forum (www.alsresearchforum.org): Online resource providing ALS researchers with relevant information to support and advance their research efforts, as well as up-to-date ALS news coverage. This unique web portal is freely available to researchers and is specifically and exclusively targeted to ALS. Some of the features available on the Forum include: research and drug development news briefs and analyses, updated listings of professional resources, including funding opportunities, jobs and scientific meetings and an exclusive database of current industry drug development efforts. The ALS Mobile Analyzer: Accessible and accurate tool for tracking ALS disease progression. P4L is developing a smartphone-based application that monitors patients in their natural environment to collect objective, ongoing, comprehensive daily-life data. The app collects data through the phone's built-in sensors and evaluates patients' functional abilities (such as speech, breathing, walking & writing) by tracking their performance of a few specifically-designed simple tasks. The app is an easy-to-use monitoring tool, freely available to all ALS patients worldwide. It will enable large scale functional data collection that will be made available for researchers worldwide via a central repository. The app will advance ALS research, facilitate individually-tailored responsive clinical care for ALS patients and will enable faster, smaller and more efficient ALS clinical trials.

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## Relevance of the methodology for analysing results – the SNIP model in amyotrophic lateral sclerosis

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Introduction: Nasal inspiratory pressure during a sniff (SNIP) is used in ALS as a complimentary test to maximal inspiratory pressure (MIP) to assess the respiratory function. It may be an alternative test to MIP in patients with bulbar involvement. Clinical relevance of variations in its technical performance is not known.
Methods: Consecutive ALS patients referred to perform respiratory tests in our Unit in January and February 2016 and age-matched healthy controls were included. All subjects were assessed in the sitting position with SNIP, performed bilaterally, with (SNIPocclud) and without (SNIPopen) closing the contralateral nostril. A minimum of 3 evaluations/nostril was done until reaching 3 consistent results. ALS patients were also assessed with revised functional ALS rating scale (ALSFRS-R) and MIP. Mann-Whitney U test and Spearmann correlation test were used to correlate variables and compare them between controls and patients. Wilcoxon test was used to compared SNIPocclud and SNIPopen in the same group of subjects. $p<0.05$ was considered as significant.
Results: Thirty-seven consecutive ALS patients and 11 controls were included. ALS patients presented significant lower SNIP values than controls, either for SNIPopen (respectively 49.4±25.2 vs 75±29.4, p=0.011) or SNIPocclud (respectively 62.8±26.8 vs 94.7±29.1, p=0.002). SNIPopen values were significantly lower both in ALS patients and controls than SNIPocclud ($p<0.001$ and p=0.007, respectively). Respectively for controls and patients, SNIPopen variation coefficient was 8.14% and 8.51%, while SNIPocclud variation coefficient was 4.98% and 6.37%. SNIPopen and SNIPocclud were strongly correlated in both cases for both groups (r=0.761 for controls; r=0.768 for patients) and moderately correlated with MIP in ALS (respectively r=0.525, p=0.006 and r=0.685, $p<0.001$). When comparing bulbar and spinal-onset patients, no differences were found for SNIPopen (respectively 42.1±31.1 vs 53.4±21, p=0.16) but the latter had significant higher values than bulbar-onset patients when performing SNIPocclud (respectively 69.1±6.1 vs 51.3±37.9, p=0.016). However, in both groups of patients SNIPocclud was significantly higher than SNIPopen(p=0.006 and $p<0.001$ for bulbar and spinal onset patients respectively).
Discussion: SNIP occluded should be considered when evaluating ALS patients as its values predict the possibility of longer follow-up periods, both in spinal and bulbar-onset patients. Further studies are needed.

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## Survival in amyotrophic lateral sclerosis and its dependency on slow and forced vital capacity

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Introduction: Slow vital capacity (SVC) and forced vital capacity (FVC) are the most frequent used tests evaluating respiratory function in amyotrophic lateral sclerosis (ALS). No previous study has determined if they equally predict survival in these patients, which is the aim of our study.
Methods: Consecutive definite/probable ALS patients (2000-2014) in whom respiratory tests were performed at baseline/ 4 months later were included. All were evaluated with revised ALS functional rating scale (ALSFRS-R), the ALSFRS respiratory (RofALSFRS-R), bulbar, upper limb and lower limb subscores, SVC, FVC, maximal inspiratory (MIP) and expiratory (MEP) pressures in addition to the King´s functional staging. SVC-FVC correlation was analyzed by Pearson product-moment correlation test. Survival analysis was done by Kaplan-Meier log-rank test and multivariate Cox proportional hazards model, with backward LP method, assessed the simultaneous effects of several independent variables on survival and adjusted the survival curves.
Results: We included 469 ALS (270 men; mean onset age 61.0 ± 11.5 years; mean disease duration 15.8 ± 16.1 months). Onset form was spinal in 329 patients and bulbar in 140. FVC and SVC were very strongly correlated ($r^2=0.981$, $p<0.001$). Significant survival prognostic variables in univariate Kaplan–Meier analysis were onset form, age at first symptoms, disease duration from symptom onset to first visit, ALSFRS and ALSFRS-R, decay of ALSFRS-R, ALSFRSb, SVC, FVC, MIP, MEP, Kings´ functional scoring, and RofALSFRS-R ($p\leq0.01$). Final Cox models with the significant variables included and either FVC or SVC showed similar results for FVC and SVC ($\beta=-0.016$; $\exp(\beta)=0.984$, 95% CI 0.978-0.99), $p<0.001$, for both) and for the other independent variables. Holding the latter constant, for every 1% decrease in the percentage of the predicted value of FVC or SVC there was a 1.02 increased probability of dying.
Conclusions: FVC and SVC are strongly strongly correlated and they are inter-changeable in predicting survival in ALS.

P30

## Existential decision-making in ALS. A comparison of legal and medical frameworks in Germany, Poland and Sweden

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Introduction: Many European countries have similar healthcare legislation concerning end-of-life decision-making and treatment provision. Despite similarities, differences exist which might be regarded minor. Systematic analyses of differences and how these impact patient's choices have been rarely studied.
Methods: We performed a systematic analysis of the legal and medical framework regulating end-of-life decisions for patients with fatal progressive disease, such as amyotrophic lateralsSclerosis (ALS) in Germany, Poland and Sweden and considered implications for a patient living in either one of the three countries.
Results: ALS-patients in Germany, Poland and Sweden are confronted with a similar spectrum of treatment options. However, the decision of whether particular life-sustaining treatments are proposed or withdrawn is made in a country-specific context. Specifically, the legal and medical frameworks differ concerning (1) the status of a patient's advance directives, (2) the preconditions for implementing life-sustaining therapies, and (3) the legal regulations on assisted dying.
Conclusions: According to the presented data, regulations of terminating life-sustaining treatments and the framework of "informed consent" is quite differently understood and implemented in the legal setting of the three countries. It is possible, and even likely, that these differences in the legal and medical frameworks have an influence on the existential decisions of patients with ALS.
This work was funded by the JPND.

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## Motor nerve biopsy in motor neuron disease and motor neuropathy: clinical, prognostic and therapeutic implications

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In order to establish the usefulness of motor nerve biopsy in the management of patients with motor neuron disease (MND) and motor neuropathy (MN), we retrospectively evaluated the clinical and pathological findings of 94 patients who underwent this procedure for diagnostic purposes. The histopathological findings were correlated with the clinical features at the follow-up to evaluate the role and accuracy of biopsy in the diagnostic, prognostic, and therapeutic assessment of MN and MND patients. On the basis of pathological criteria motor nerves were classified into five groups: suspected MND; suspected MN; final etiological diagnosis; uncertain diagnosis; not diagnostic. Moreover, diagnostic biopsies were further classified into acute or chronic, according to pathologic findings. A total of 76 (80.85%) clinical follow-up were available for comparison with pathological results. In these patients, a pathological diagnosis was performed in 53 (69.74%) cases of which MND in 35 patients (46.05%) and MN in 15 (19.73%). In three cases the biopsy revealed a final etiological diagnosis (5.66%). In the remaining 23 patients (30%) of the 76 patients with follow-up, motor nerve biopsies were not conclusive. A definitive LMND and MN diagnosis was confirmed at follow-up in 33 (94.29%) and 15 (100%) cases respectively. On the basis of the pathological diagnosis, treatment has been performed in all patients that was beneficial at the follow-up in 58.82% of the MN cases. Our data confirm that motor nerve biopsy is a reliable method for the initial evaluation and differential diagnosis of MN and MND in selected patients providing prognostic/therapeutical information, particularly for MN patients that may undergo specific therapy. Finally, motor nerve biopsy may help to investigate disease mechanisms in MND.

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## In ALS patients hypoventilation and cough inefficacy correlate with functional performance decline on the 6MWT

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Introduction: ALS is a progressive neurodegenerative disorder and respiratory failure is the first cause of death. Six minutes walking test (6MWT) is usually performed to assess exercise capacity. Nowadays, few studies examined how the most common respiratory parameters correlate with functional parameters.
Aim: To evaluate the relationship between respiratory measures and 6MWT parameters in an ALS population, and examine its trend in a 6-months follow-up period.
Methods: Lung and motor functions were assessed in all patients at the first evaluation in our Center (T0) and after 6 months (T1), with sitting and supine spirometry, measurement of peak expiratory cough flow (PCF), nocturnal pulse oximetry, arterial blood gases analysis, and 6MWT. We studied correlations among sitting (FVCorto%) and supine (FVCclino%) forced vital capacity, PCF, mean noctural SpO2, oxygen desaturation index (ODI), pH, pO2, pCO2 and HCO3-, and variables measured during 6MWT: mean distance in meters (Dm) and %theoric (D%), SpO2 at rest, SpO2 during the test, SpO2 nadir, Borg dyspnea at rest (BDpre) and at the end of the test (BDpost). In order to assess if the trend of respiratory and motor function were correlated, we analyzed their decline.
Results: 26 patients (mean age:60.2 ±9.67 y; M/F ratio:20/6) were studied. At T0 no correlation was found between the investigated variables. At T1 we detected direct significant correlations between FVCclino% and PCF, and Dm and D% (r=0,58; r=0,54 and r=0,60; r=0,51, respectively) and between pCO2 and BDpost (r=0,62). Only one inverse correlation between HCO3- and mean SpO2 during the test was found (r=-0,60). The longitudinal data analysis between T0 and T1 showed direct correlations between pCO2 and BDpost variations (r=0,61), and between FVCclino% and mean SpO2 during the test variations (r=0,61). An inverse correlation was found between HCO3- and both Dm and D% (r=-0,57; r=-0,64, respectively).
Conclusion: In conclusion, our data shows that hypoventilation onset and reduction of cough efficacy are linked to the decline of functional performances on the 6MWT, with reduction of the covered distance, worsening of dyspnea during effort and of oxyhemoglobin saturation during the test. Therefore, our preliminary data prove that, in ALS, motor performance is strongly correlated with respiratory performance.

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## End-of-life decisions in ALS: from physicians' perspective

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Along with disease progression, patients with amyotrophic lateral sclerosis (ALS) are confronted with end-of-life decisions. They may decide for or against life-prolonging methods, including enteral nutrition (EN), non-invasive ventilation (NIV), invasive ventilation (IV). Depending on the local law, they may also consider life-shortening methods such as continuous deep sedation (CDS), physician-assisted suicide (PAS) and euthanasia. The aim of this study was to assess the attitude of the Polish neurologists to the end-of life decisions in patients with ALS.
Method: A group of randomly selected Polish neurologists (n=431) were asked to fill in an anonymous questionnaire, which consisted of 47 questions considering own experience and personal attitude towards the life prolonging and life-shortening methods. The study was performed between June 2016 and March 2017. The response rate was 13.7% (n=59). Seventy five percent of inquired neurologists had an over 10 year-long clinical experience.
Results: In the opinion of physicians, the life prolonging methods (EN, NIV, IV) are beneficial for ALS patients in 96.30%, 90.74%, and 62.96%, respectively. The neurologists have a positive personal attitude towards the above-mentioned methods in 94.3%, 96.23% and 52.83%, respectively. Interestingly, only 41.5% of physicians would decide to prolong their own lives with IV in case they had medical indications for its use. While neurologists commonly discuss life-prolonging methods with ALS patients (96.23%), life-shortening methods are only discussed when particularly asked for it (39.6%) or at the advanced disease stage (18.86%). Only 32.1% of neurologists report ever being asked by ALS patients to introduce life-prolonging treatments, 13.2% to withdraw them, while only 1.9% were asked to introduce life-shortening methods. Of all responders, 22.6% would consider implementation of life-shortening methods if asked by an ALS patient, while 77.6% of physicians would not consider them due to: 1) ethical, 2) legal, 3) religious, 4) personal or other reasons.
Conclusion: The physicians' attitude towards end-of life decisions in ALS patients mostly depend on the current treatment and legal recommendations. Their personal attitude may however influence the subject choice in discussions with patients.

P38

## Does wellbeing of patients with amyotrophic lateral sclerosis depend on their population of origin?

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Introduction: In a recent European study [in press], Polish patients were found to have a significantly lower QoL and a higher depression rate as compared to German and Swedish individuals. They were also characterized by a higher religiousness and lower autonomy. The aim of the study was to analyze whether the observed changes are related to the ALS patients' conditions (economic/medical/social care) in the country of origin, or if they depend on a reduced perception of the actual well-being characterizing the Polish society.
Material and Methods: 60 Polish patients with ALS (age 29-87, mean 55±12.27, 58.33% males), and 60 healthy controls matched for age, gender and education status (age 29-84, mean 55±12.83, 58.33% males) were included in the study. The subjective and global QoL was assessed by Schedule for the Evaluation of Individual Quality of Life – Direct Weighting (SEIQoL-DW) and the Anamnestic Comparative Self Assessment (ACSA), respectively. We also analyzed the ALS-Depression-Inventory (ADI-12), the Idler's religiosity scale (IIR) and the Shared Decision Scale (SDS).
Results: The Polish patients had a significantly lower subjective and global QoL compared to healthy controls (SEIQoL-DW 66.21 vs 73.72 and ACSA 4.63 vs 6.57, both $p<0.05$). Both ALS patients and controls focused on similar domains: family (19.33/17.33%), psychosocial/existential factors (15.67/21.00%), health (14.67/17.33%) and recreation (13.33/19.00%). Compared to healthy controls, ALS had a negative impact on health, recreation and, psychosocial/existential domains. The depression rate was similar in both groups (24.18 vs 23.67). ALS patients considered themselves less religious than healthy controls (88.34% vs 91.67%) but the differences were not significant. The ALS patients also had lower preference to have physician dominate in medical decision-making (30% vs 70%).
Conclusions: We have demonstrated a significantly reduced QoL in Polish ALS patients compared to controls. The results in both groups were however lower than those obtained in German and Swedish patients what suggests a different basic perception of QoL in the Polish society. Also the depression rate was comparable between the Polish patients and controls, which suggests that an overall analysis of well-being should take into account the population-based determinants.

**P40**

## Amyotrophic lateral sclerosis domain ontology (DALSO) – first results

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The work presents ALS ontology, semantic model of domain specific knowledge related to this disease. DALSO ontology is based on standardized domain terminology, expressing disease related knowledge in a formal, machine-readable format, allowing for further processing. Medical data needed for reliable ALS diagnosis represented in DALSO ontology contain results from different medical knowledge areas such as: patient demographic and life style, neurological symptoms and clinical findings, wide range of investigations including clinical electrophysiology, neuroimaging data, laboratory and genetic tests, medical history of a patient (e.g. comordibities) and his/her family medical history (ALS and other motor neuron disease), ALS type classifications and clinimetric scales for quantitative assessment of patient functional or mental state. At current stage of ontology development granularity of represented knowledge is tailored to the needs of the project ONWebDUALS, while the scope of knowledge in selected modules exceeds the range needed in the project. Granularity of domain knowledge and its scope can be enlarged and used in other ALS related projects if needed. The medical knowledge related to ALS in DALSO ontology is organized into 14 orthogonal, i.e. independent modules, formed by sets of homogenous class hierarchies, joined with class definitions used for restrictions and complex notions descriptions. In order to standardize ALS related terminology and facilitate integration with other ontological models, most of relevant DALSO concepts are annotated with identifier from top-level reference models such as Human Phenotype Ontology, LOINC ontology, Disease ontology and Foundational Model of Anatomy. Salient ontology concepts are also annotated with human readable textual explanations.
Conclusion: DALSO ontology contains at the moment 897 classes, 258 properties (123 objects, 135 data types). The model is consistent and free of logic errors, its syntactic correctness was confirmed by Fact++ reasoner embedded in Protégé-2000 ver. 4.3 ontology editor used to develop the model. DALSO ontology is expressed in Ontology Web Language version 2 [OWL2], because it provides the maximum expressivity and high computational capacity.

**P42**

## A conceptual multi-modal cognitive model of affective social cognition (MASC) in amyotrophic lateral sclerosis

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Cognitive impairment is an integral part of amyotrophic lateral sclerosis (ALS), and social cognitive processes are known to be impaired in some patients. Social cognition refers to the specialized domain of cognition that underlies mental representations of the self, others, and the social world. Social cognition is not a unitary process, but one which depends on the operation of potentially independent, yet interactive, components. To date, no clinically robust cognitive model of social cognition exists.
Method: A population-based cohort patients were recruited to this on-going study of cognitive heterogeneity in ALS (N=123). Healthy controls were also recruited to provide matched reference data (N=110). Patients and controls completed a comprehensive battery of neuropsychological tests, with the inclusion of social cognitive measures.
Results/Discussion: These data integrate a bottom-up approach to social cognition which propose that lower level base functions (modules) are necessary to support the development of higher order social cognitive functions (a central system). Outcome measures are discussed in relation to developing a cognitive model of social cognition, considering modularity, universality, and subtractivity within the context of neurodegenerative conditions.

P44

## Digit Span Forward, a brief and simple test useful for predicting prognosis in ALS

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Amyotrophic lateral sclerosis (ALS) is a multisystem disorder characterized by the degeneration of upper (corticospinal) and lower (spinal and bulbar) motor neurons, leading to progressive atrophy and paralysis. In addition, a spectrum of cognitive and/or behavioural dysfunctions constitutes the non-motor manifestations. Determining the spectrum of cognitive and functional abnormalities may help understand the clinical implications in ALS, providing indications on the etiology of neurodegeneration. The aim of this study was to assess the effect of cognitive dysfunction on ALS survival and to investigate the use of neuropsychological measurements as tools for predicting a poor prognosis in ALS. The retrospective sample included 76 ALS patients, 52 men and 24 women with a mean age at evaluation of 61,12 (SD 12,71) years, attending the inpatients and outpatient facility at NEMO (NEuroMuscular Omnicenter) between 2011 and 2016. Diagnosis of possible, probable or definite ALS was defined according to the revised El Escorial criteria. The median survival time from onset to death was 31 months. According to univariate analysis, factors related to survival from onset to death were used in the multivariable analysis, adjusting for the significant explanatory variables, and Digit Span Forward resulted as the only independent prognostic factor of survival in ALS (HR=0.302, p= 0.0272), demonstrating that ALS patients that performed poorly in the Digit Span Task had a shorter life expectancy. Digit Span Forward is a rapid, feasible and reliable assessment tool, providing an effective measure for executive impairment detection in ALS patients and more in general in neuronal degeneration. Since Digit Span Forward provides a dual mode of execution, accommodating both verbal and motor disabilities, it fits the ALS clinical spectrum. In the present study, Digit Span Forward has shown to be sensitive in detecting early degeneration in prefrontal regions and in predicting poor survival in ALS patients.

**P46**

## A secondary analysis of the Reading the Mind in the Eyes Test within ALS: the development of a rapid and reliable short form

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Executive dysfunction is common in patients with ALS, with up to 50% of patients performing within an impaired range. There is evidence that social cognitive deficits associated with ALS are a function of deficits in executive function. A recent study by this group evidenced the 'Reading the Mind in the Eyes' Test (RMET) as a recognized test of social cognitive function in ALS. This recent study of this measure within ALS provided a Cronbach's Alpha of .73, indicating good reliability. Split-half reliability analysis further confirmed these findings ($p= 0.826$). The Reading the Mind in the Eyes test had excellent psychometric properties when discriminating between ALS patients who are cognitively intact, and those who have executive impairment, with an overall medium difficulty. There was a large magnitude significant difference between patients and controls ($p< 0.001$; $\eta^2= .19$). Post-hoc analysis revealed that controls performed significantly higher than patients with executive impairment ($p< 0.001$), and patients with single executive deficits ($p= 0.002$). The present study aims to provide a secondary analysis of these data, to determine whether the RMET is a valid tool as a 'short-form', using 18 of the original 36 items. This study contributes not only to the psychometric knowledge of this measure, but also to the usability, effectiveness, efficacy, and reliability of social cognitive assessment in ALS. Furthermore, if found reliable, this measure may be a tool which could be employed in clinic-based settings for a rapid and reliable assessment of social cognition in ALS.

**P48**

## Is there an association between attention-deficit/hyperactivity disorder (ADHD) and amyotrophic lateral sclerosis (ALS)?

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Introduction: Attention-deficit/hyperactivity disorder (ADHD) belongs to the most common infantile neuro psychiatric diseases. It is assumed that the prevalence in children and adolescents is 4%, about 30-60% persist into adulthood. Similarities in phenomenology and neurobiology of ADHS and ALS such as increased occurrence of head injuries, deficits in frontal executive functions and comparable neurobiological changes in glutaminergic and dopaminergic activity let to the hypothesis, that there could be a link between the two diseases (Lulé D. et al. (2008)). We aimed to investigate if patients diagnosed with ALS have a higher prevalence of ADHD in childhood and in adulthood.
Methods: We used standard questionnaires to assess childhood and adult ADHD; the German short version of the Wender Utah Rating Scale (WURS-k) to retrospectively rate ADHD symptoms in childhood and the ADHD self-rating scale (ADHD-SR) to assess current ADHD symptoms. A representative sample of the general population and a sample with Parkinson's disease (PD) served as clinical control groups. We used binary logistic and linear regression analyses with adjustment.
Results: No differences were found between ALS and PD patients and the general population with regard to the prevalence of adult ADHD. Overall, the prevalence rates in both clinical samples were very low. The prevalence of childhood ADHD only was significantly lower in the ALS group but not in the PD group compared to the general population. With regard to the individual ADHD symptom scores, patients with ALS reported significantly lower childhood ADHD scores and lower attention deficit scores in adulthood compared to the general population. These differences were also shown between PD patients and the general population.
Conclusion: Contrary to our hypothesis, patients with ALS exhibited significantly lower rates of self-reported childhood ADHD and no difference in adult ADHD rates compared to the general population.

**P50**

## Preliminary evaluation of hopelessness and depression in patients with amyotrophic lateral sclerosis undergoing a Mesenchymal Stem Cell clinical trial

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Background: This preliminary study aims to analyze hopelessness and depression in patients with amyotrophic lateral sclerosis (ALS) undergoing a Mesenchymal Stem Cell (MSC) clinical trial before, in the period of and after 2 intrathecal cell injections, in order to evaluate psychological aspects regarding the treatment.
Methods: Hopelessness and depression are being evaluated in ALS patients undergoing a two intrathecal MSC injections with a 30 days-interval. Up to date, patients have been evaluated for 3 and 2 months, after the first and second injections, respectively. The Beck Hopelessness Scale (BHS) is a dichotomous instrument with 20 items, wich measures pessimism and offers evidence of suicide risk in depressed subjects. The score of hopelessness ranges from 0-4 (Minimum), 5-8 (Mild), 9-13 (Moderate) and 14-20 (Severe). The ALS-Depression-Inventory (ADI-12) is an instrument composed by 12 statements with 4 possible answers: 'I fully agree', 'I agree', 'I don't agree' and 'I do not agree at all'. It has 4 positive and 8 negative affirmatives. The scores between 23–29 indicate mild depression and scores above 30 show severe depression.
Results: 46.15% patients informed the use of depression/anxiety medicine. None of them claimed history of suicidal ideation, but thoughts about death and future were reported. At the time of screening, the BHS and ADI-12 indicated a predominance of minimum hopelessness (85.8%) and mild depression (57.2%). At time of first infusion, ALS patients showed the highest score of minimal hopelessness (100%), a decreasing score of mild hopelessness to 0% and mild depression to 50%. At the day of infusion 2, the minimum hopelessness score decreases (80%) while the mild one was elevated about 20% and the mild depression score decreased about 30%. One month after second infusion, the minimal hopelessness score decreased (66.7%), the mild hopelessness was elevated (33.3%) and the mild depression increased about 13.3%. However, two months later, the mild depression score reached 100%, while the minimal hopelessness (66.7%) and the mild hopelessness (33.3%) remained unchanged.
Conclusion: The BHS and ADI-12 indicated the maintenance of hope through up the study with some indications of the presence of an increased depression with the progression of the disease.
Funding: This work was supported by FAPESP and CNPq, Brazil.

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## Investigation of new generation sequencing technologies and varian calling methods on Whole Genome sSequencing and Miseq data of ALS Patients

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Next generation sequencing (NGS) is being used to investigate the genetic bases and causes of ALS as well as to identify novel therapeutic targets. There are a number of NGS service providers. Among these, illumina has a leading role and its Whole Genome Sequencing (WGS) service and Miseq platform are widely used. Big international projects such as ProjectMinE are using illumina's WGS service for sequencing tens of thousands of genomes, guaranteeing high and deep coverage data. In addition, the illumina Miseq platform is becoming the leading technology for high quality sequencing of targeted regions of the genome. Indeed, MiSeq's feasibility as a potential diagnostic tool for patients with ALS was recently demonstrated. In this project we want to investigate the strengths and weaknesses of these two NGS techniques (WGS vs MiSeq) and assess the performance of the available state of the art methods for variant calling on a dataset of 95 ALS human samples sequenced both with WGS (coverage depth ~40x) and Miseq (~1000x) technologies. A panel of 25 ALS-related genes was used for the Miseq platform. To this aim, a set of analyses were performed focusing on both the quality of the sequencing data sets and the variants called by six state of the art variant callers (VCs). The illumina Infinium Omni2.5-8 BeadChip (IOC) genotyping of 2.5 million common variants was used as a set of true positives for the assessment of SNP calling. On the gene panel the WGS data presented a coverage of at least 30x for 89% of the targeted regions, while the Miseq data could guarantee 30x coverage for 87% of the target genes. On average, the VCs performed better on the WGS data in terms of both precision (~94%) and sensitivity (~90%), when compared with the IOC genotyping. This gives insight into the realibility of the WGS data which is of crucial importance for many genetic initiatives wolrdwide. It also promotes such technology as a valid tool for medical application. Among the VCs, Freebayes and Vardics performed comparably with the highly rated GATK-HC despite requiring much shorter computational time. When the variants called on the Miseq and WGS data were compared, we observed an unexpected low concordance (~75%) considering the very high precision and sensitivity that the variant callers showed in the previous analysis. Variant filtering was able to increase their concordance up to 80% without compromising the overall precision and sensitivity.

**P54**

## CHCHD10 variants in amyotrophic lateral sclerosis: where is the evidence?

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Objective: After the initial report of a CHCHD10 mutation in mitochondrial disease with features resembling amyotrophic lateral sclerosis (ALS), CHCHD10 mutations have been considered to be a frequent cause for ALS. The exact pathogenicity and clinical significance, however, of these mutations remain unclear. We aimed to determine the role of CHCHD10 mutations in ALS.
Methods: We included 4,365 whole genome sequenced ALS patients and 1,832 controls from 7 different countries and examined all non-synonymous single nucleotide variants (SNVs) in CHCHD10. These were tested for association with ALS, independently and in aggregate using several genetic burden tests (including SKAT and SKAT-O).
Results: We identified three new variants in cases, but only one was case-specific. Also, one control-specific mutation was identified. There was no increased burden of rare coding mutations among ALS patients compared to controls (P = 0.88 and P = 1.00 for SKAT and SKAT-O, respectively). The few carriers with potential pathogenic CHCHD10 mutations exhibited a slowly progressive ALS-like phenotype with atypical features such as myopathy and deafness.
Interpretation: CHCHD10 mutations seem to be a far less prevalent cause of pure ALS than previously suggested, but instead appear related to more complex phenotypes. There appears to be insufficient evidence for the pathogenicity of most previously reported variants in pure ALS. This study shows that routine testing for CHCHD10 mutations in pure ALS is not recommended and illustrates the importance of sufficient genetic and functional evidence in establishing pathogenicity of genetic variants.

**P56**

## Unexpanded C9ORF72 alleles are not ALS risk factors

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Introduction: Amyotrophic lateral sclerosis (ALS) is one the most severe motor neuron (MN) disorder in adults. The C9ORF72 repeat expansion is the most common genetic cause to the disease. Initial findings have set the pathogenic cutoff to 30 repeat. However, intermediate repeat number between 16 and 30 have also been proposed to be associated with ALS risk. As most studies rely on ALS patients, we performed a case-control study in a French cohort to precise the involvement of intermediate repeats on the ALS risk.
Methods: In a cohort of 412 C9ORF72-negative sporadic ALS patients and 327 healthy controls, the C9ORF72 repeat number was assessed by repeat-primed PCR.
Results: The most frequent alleles were 2, 5 and 8 repeats both in ALS and control groups. The highest repeat number was 22 in controls and 26 in patients. The allelic distribution was not significantly different between both groups.
Conclusions: These findings show a lack of association between C9ORF72 intermediate repeat numbers and the risk of developing ALS. This data suggest that repeat numbers below 30 is not an ALS risk factor in French population and confirms the definition of the C9ORF72 pathogenic cutoff.

**P58**

## ALS in Turkey: recent insights from genetic studies

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Turkey, with its wealthy historical background and unique geograph, forms a natural bridge between Europe and Asia. The coastal areas at the Mediterranean Sea and extensive rural parts bordering the Near Eastern countries and the Black Sea make Turkey a rich genetic pool with a high ethnic heterogeneity. In contrast with other European populations, in which family sizes have been decreasing steadily in the last 50 years, Turkey is still very dynamic, with high birth rates and traditionally large kindreds consisting of several living generations and an impressive number of offspring. Because close consanguineous marriages are still part of the Turkish culture, exceeding 60% in the eastern parts of the country, the number of autosomal recessively inherited forms of diseases is in excess of what is to be expected. In this study, we report the results of our systematic research on the molecular basis of ALS in Turkey. We screened 890 ALS patients for the most common genes, including 230 fALS patients (171 families) and 660 sporadic cases. C9ORF72, SOD1, FUS, TARDBP, UBQLN2 mutations together account for 34% of fALS in Turkey. Exome analysis in consanguineous families reveals recessive mutations in diverse genes implicated also in other disorders: OPTN, SPG11, DJ1, PLEKHG5, SYNE1, TRPM7, SQSTM1, C19ORF12, DNAJB2, ERLIN1, IGHMBP2 and SLC12A6, many being novel; dominant mutations in VCP, ERBB4 and ANG genes were also shown. Currently, 313 ALS patients and 113 controls are being processed in the framework of Project MinE. The epidemiology of ALS in Turkey has features representing the pattern seen in Caucasian populations; however, it has also specific aspects, such as the more complex nature of the disease in molecular and clinical terms. The rich spectrum of mutations reflects both the different genetic background and the heterogeneous nature of the Turkish population, broadening the phenotype associated with ALS.

**P60**

## SMN2 gene copy number and promoter methylation as disease modifiers of childhood-onset spinal muscular atrophy

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Introduction: Spinal muscular atrophy (SMA) represents the most frequent monogenic cause of infant mortality. Degeneration of lower motor neurons and progressive muscle weakness are caused by homozygous absence of the survival of motor neuron 1 (SMN1) gene. Clinical presentation of SMA is extremely variable ranging from a fatal infantile form (type I) to adult-onset form (type IV). Homogeneity of the disease-causing mutation and extensive phenotypic variability clearly indicate the existence of additional (genetic, epigenetic and environmental) factors modulating disease progression. The SMN2 gene copy number is considered to be a major genetic modifier of SMA, although patients with equal SMN2 gene copy number may display different clinical outcome.
Objectives: We aimed to examine the effect of the SMN2 gene copy number on phenotypic variability of childhood-onset SMA, as well as levels and pattern of methylation of the SMN2 promoter-associated CpGs, including those co-localising with the transcriptional start sites of SMN2 at positions −296 and −290.
Material and methods: MLPA was used to assess the SMN2 gene copy number in 99 genetically confirmed Serbian SMA patients (23 with severe type I, 37 with intermediate type II and 39 with mild type III). Bisulphite PCR amplification followed either by methylation-sensitive high-resolution melting analysis (MS-HRM) or cloning and Sanger sequencing of positive clones were used to analyse levels and pattern of methylation of SMN2 promoter-associated CpGs.
Results: Inverse correlation was observed between the SMN2 copy number and SMA type (Spearman rank test, p=2.2e-16). Complex and uninterpretable melting curves obtained by MS-HRM incomparable with those derived from standards with a known methylation level pointed towards heterogeneous SMN2 promoter methylation in our patient samples. Analysis of one type I SMA patient and one type III carrying equal SMN2 copy numbers by cloning and Sanger sequencing confirmed presence of multiple and extremely heterogeneous epialleles.

Conclusion: SMN2 gene copy number modifies childhood-onset SMA clinical outcome among Serbian patients. The role of SMN2 promoter methylation in modulating SMA phenotype could not be elucidated using MS-HRM analysis due to heterogeneous DNA methylation, and this issue should be further studied by targeted DNA methylation analysis by next-generation sequencing.

**P62**

## First report of angiogenin gene mutations in patients with amyotrophic lateral sclerosis in Serbia

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Mutations in angiogenin gene (ANG) have been associated with amyotrophic lateral sclerosis (ALS) in several populations but systematic study of ANG mutation prevalence and phenotype in Serbian ALS patients has not been performed so far. In our study, comprehensive mutation screening of coding regions of ANG gene was performed in 234 Serbian ALS patients. All patients had been also screened for SOD1, C9orf72, FUS (exons 14 and 15) and TDP-43 (exone 6) mutations and 214 patents were negative. Three previously described missense changes in ANG gene were identified in four cases. Two substitutions are affecting mature protein (K17I and I46V), and one is affecting signal peptide (M-24I). Two of our patients had single mutation and one was carrier of two changes in ANG gene. Fourth patient had triple mutation in c9orf72, FUS and ANG genes. We found atypical signs in ANG positive patients, such as Parkinsonism and FTD. In line with this, ANG mutation have previously been described in these degenerative disorders. Mutation frequency in our study was 1,4%, so we can conclude that mutations in ANG gene are not the common cause ALS in Serbian population. Substitution in signal peptide (M-24I) was, according to our knowledge, previously described in Italian ALS patients, so estimation of mutations frequency and type in Serbian ALS patients is important for efficient genetic testing strategy.

P64

## Genetic heterogeneity of amyotrophic lateral sclerosis in the Hungarian population

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Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease associated with degeneration of upper and lower motor neurons. Affected individuals usually die of respiratory failure within 3-5 years. Genetic factors play a key role in all types of ALS, therefore, the goal of the project was to investigate Mendelian-disease causing genes in order to find causative mutations.
Patients and methods: The investigated sporadic patients (n=70) fulfilled the revised El Escorial and the Awaji-shima criteria for ALS. Using Sanger sequencing, we screened mutations in SOD1, ANG, TARDBP and UBQLN2 genes in 70 Hungarian patients. A two-step amplicon fragment length analysis protocol was followed for the detection of the repeat expansion in the C9ORF72 gene in all patients. Genotyping was used to determine whether the individual carrying the repeat expansion carried the "risk" haplotype. Targeted high-thoughput sequencing was used to establish frequencies of mutations in the coding regions of the SETX, FUS and C9ORF72 genes in patients who did not carry mutations in the SOD1, ANG, TARDBP and UBQLN2 genes.
Results: We identified a novel frameshift (K91RfsTer8) and three known missense mutations (V14M, L144F and D90A) in the SOD1 gene in five patients. The novel mutation led to a frameshift with the insertion of 8 novel amino acids and the formation of premature stop codon. Mutation analysis of the TARDBP gene revealed a recurrent missense mutation (M311V) in a sporadic patient. Two novel (A55P; R33W) and two known mutations (M-24I; V103I) were detected in the ANG gene in five patients. C9orf72 repeat expansion was identified in one patient, who also carried the rs3849942 risk allele. High-troughput sequencing identified an additional novel missense mutation (N264S) in the SETX gene.
Conclusion: We performed the first genetic analysis in a cohort of Hungarian ALS patients. Disease causing variants have been detected in approximately 17% of this sporadic cohort. Patients in whom the targeted sequencing approach failed to detect disease causing mutations are being investigated by whole exome sequencing. Our study adds novel data to the genetic diversity of ALS and indicates that complex approaches are needed to understand the genetic heterogeneity of this disease.

P66

## Age and education – protective factors against cognitive decline in ALS indicated by intrinsic functional connectivity patterns

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Next to motor disabilities 30-50% of ALS-patients also present with cognitive impairment. Although these impairments have been investigated thoroughly, little is known about their cortical signature measured with intrinsic functional connectivity (IFC) of resting-state-networks (RSN) and possible protective factors. There is evidence from literature that cognitive performance in ALS is correlated with changed IFC of the frontal cortex. Moreover, age and education are known to have a protective effect on cognitive performance in ALS. This study therefore investigated the IFC of N = 57 ALS-patients, measured with functional magnetic resonance imaging (fMRI). Two RSNs, namely the Motor- (MN) and Default-mode-network (DMN) were observed and associated with age and education in addition to cognitive performance, assessed by the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). Analysis was performed with the open-source software DPARSF. Concerning cognitive performance significant negative correlations were observed in the frontal, parietal and motor cortex within the MN and DMN. Significant positive correlations between age and both RSNs were found in areas involved in motor and cognitive control. Moreover, significant negative correlations were found between years of education and the DMN. Results implicate an increase of IFC with decline in cognitive performance in patients with ALS which is counteracted by young age and higher education. These can most probably be considered to be protective factors in the course of cognitive decline in neurodegeneration in ALS. Similar patterns have been reported for other neurodegenerative processes associated with cognitive decline such as in Alzheimer's disease.

**P68**

## Imaging profiles of ALS-FTD cohorts: looking beyond C9orf72

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Background: The clinical, pathological and neuropsychological overlap between amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD) is well established. Since the discovery of the hexanucleotide repeat expansion in C9orf72 in 2011 the majority of neuroimaging studies have focused on the characterisation of ALS-FTD patients carrying the repeat and relatively little is known of the imaging profile of ALS-FTD patients who don't carry the hexanucleotide expansion. The objective of this study is to comprehensively characterise and compare the neuroimaging profiles of C9orf72 positive and negative ALS-FTD patients.
Methods: Ten patients with ALS-FTD carrying the C9orf72 hexanucleotide repeat (C9+ ALS-FTD), ten patients with ALS-FTD without the C9orf72 repeat (C9- ALS-FTD) and twenty cognitively normal ALS patients (ALSnci) were included in a prospective quantitative neuroimaging study. All patients tested negative for a comprehensive panel of other genes implicated in both ALS and FTD. Cortical grey matter morphometry analyses were performed using both a whole-brain and region-of-interest approach. Multiparametric diffusion tensor imaging (DTI) analyses were carried out to characterise genotype-specific white matter degeneration using axial diffusivity (AD), mean diffusivity (MD), radial diffusivity (RD) and fractional anisotropy (FA).
Results: Compared with the cognitively normal ALS group, grey matter abnormalities were more widespread in C9- ALS-FTD group than in the C9+ ALS-FTD group. Irrespective of the genotype, ALS-FTD patients showed extensive extra-motor white-matter pathology in comparison to the cognitively normal ALS group. Interestingly, ALS-FTD cohorts also exhibited marked motor cortex and corticospinal tract pathology compared to ALSnci patients. FA and RD changes in orbitofrontal and pre-central regions were more pronounced in C9- ALS-FTD group than in the C9+ve ALS-FTD group. AD changes were less extensive overall than other indices although once again changes were more apparent in the in C9- ALS-FTD group.
Conclusions: Our study serves as a reminder that C9orf72 hexanucleotide expansions do not account for all cases of ALS-FTD. While C9 negative ALS-FTD patients also exhibit catastrophic motor and extra-motor degeneration, their genetic susceptibility remains poorly understood.

P70

## Relationship between brain metabolism and cognitive/behavioral functioning in ALS

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Aims: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, with a median survival of 33 months. A strong clinical and molecular link between ALS and the neurodegenerative disorder frontotemporal dementia (FTD) has been uncovered. About 10% of ALS patients develop co-morbid FTD (ALS-FTD) and up to 30-40% display mild cognitive or behavioral impairment. The aim of this study was to relate a range of cognitively and/or behaviorally impaired ALS patients to regional brain glucose metabolism.
Methods: 75 ALS patients were subjected to the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) and 18F-FDG PET/CT. According to their ECAS performance, patients were divided into three groups: cognitively normal patients (ALS-CN), patients with cognitive or behavioral impairment (ALS-Ci/Bi) and patients with ALS-FTD. Static 18F-FDG PET images were acquired 30 min after injection of 150 MBq 18F-FDG. PET images were analyzed using a VOI-based (Hammers atlas, PNEURO v3.6) and a voxel-based (SPM8) approach. 18F-FDG uptake was normalized to the average whole-brain grey matter uptake. Correlations between regional 18F-FDG uptake and ECAS performance were assessed using linear regression analyses and group analysis, the level of significance was set at $p < 0.05$ and Bonferroni correction was performed to correct for multiple testing.
Results: A voxel-wise group comparison revealed significant relative hypometabolism in the middle frontal gyrus and subcallosal area of ALS-FTD patients when compared to ALS-CN patients (cluster-level threshold of PFWE-corrected < 0.001, T=3.3, kE = 20 voxels). VOI-based correlation analysis displayed a positive correlation between ALS-specific ECAS score and 18F-FDG uptake in the frontal lobe ($r = 0.50$, $p < 0.001$), the prefrontal cortex (PFC) ($r = 0.50$, $p < 0.001$) and the cingulate cortex (CC) ($r = 0.49$, $p < 0.001$). 18F-FDG uptake in the frontal lobe ($r = 0.41$, $p < 0.001$) and PFC ($r = 0.40$, $p < 0.001$) also correlated positively with verbal fluency and tracer uptake in CC with executive functioning ($r = 0.41$, $p < 0.001$).
Conclusion: Poor ECAS performance, is associated with relative frontal hypometabolism. These results extend previous findings that 18F-FDG PET could be an early marker for cognitive and behavioral impairment in ALS.

**P72**

## Spinal cord multi-parametric MRI for survival prediction in ALS

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Background: The need for useful biomarkers predictive of survival is still unmet in ALS but new developments of spinal cord (SC) imaging seem very promising. Moreover, our previous findings suggest strong relationship between SC atrophy and disease progression (1). Aim of this study was to determine if multimodal MRI of the SC could be predictive of survival in ALS.
Methods: 49 ALS patients were recruited and clinical data collected. Patients were scored on ALSFRS-R and manual muscle testing. They were followed longitudinally to assess survival. Cervical spinal cord was imaged using 3T MRI system. Cord volume and cross-sectional area (CSA) at each vertebral level were computed. DTI metrics were measured. Imaging metrics and clinical variables were used as inputs for a multivariate Cox regression survival model.
Results: When building a multivariate Cox regression model with clinical and MRI parameters, FA, MTR, and CSA at C2-C3, C4-C5, C5-C6 and C6-C7 vertebral levels were significant. Moreover, hazard ratio (HR) calculated for CSA at C3-C4 and C5-C6 levels indicated an increased risk for patients with SC atrophy (respectively 0,66 and 0,68). In our cohort, MRI parameters seem to be more predictive than clinical variables, which had HR very close to 1.
Conclusions: Our data suggest that multimodal SC MRI could be a useful tool in survival prediction especially if used at the beginning of the disease and when combined with clinical variables. To validate it as a biomarker, results confirmation in independent bigger cohorts of patients is warranted.

**P74**

## Modelization of intrinsic motor neuron defects in amyotrophic lateral sclerosis with human induced pluripotent stem cells

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ALS involves motor neuron death and today mechanisms underlying this selective motor neuron loss remains unclear. In order to analyze the contribution of ALS causing mutations directly in human motor neurons, we have generated induced pluripotent stem cells (iPSc) from ALS patients carrying mutations in the three main genes responsible for ALS (C9ORF72, SOD1, TARDBP) and patients with sporadic forms, and produced human iPSc-derived motor neurons in high proportions. Whereas most published reports study patients with one specific ALS form, our project aims to compare different ALS forms in a same experimental context in order to identify similarities and differences between the different forms. Electrophysiological recordings at two time-points of motor neuron cultures suggest that the excitability of the ALS-derived motor neurons is altered differently in motor neurons derived from mutant SOD1 and mutant C9ORF72 iPSc than those derived from mutant TARDBP iPSc. Analysis show that ALS motor neurons cultured in the presence of neurotrophic factors survive in culture like control ones. However, preliminary data suggest that under stress like neurotrophic factor deprivation, mutant SOD1 and C9ORF72 motor neurons show growth defects and mutant SOD1 and TARDBP motor neurons degenerate. Furthermore, mutant SOD1 and C9ORF72 motor neurons accumulate neurofilament inclusions but not motor neurons carrying TARDBP mutation. Interestingly, these accumulations are localized in the axonal initial segment (AIS), important for maintenance of axonal identity and firing action potential. Taken together, these results suggest that different pathways to degeneration may be followed in ALS motoneurons carrying different mutations and draw the hypothesis of a perturbation of the AIS as a link to motor neuron defect in ALS.

**P76**

## Motor neurons derived from induced pluripotent stem cells of patients with mutations in TARDBP show axonal transport defects

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TAR DNA binding protein 43 kDa (TDP-43) is a major component of pathological inclusions in sporadic and familial amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U). ALS patients suffer from progressive degeneration of motor neurons, while FTLD is characterised by the progressive degeneration of cortical neurons in the frontal and temporal lobe. While hyperphosphorylated and ubiquitinated TDP-43 inclusions are a pathological hallmark of both ALS and FTLD-U, mutations in the gene encoding TDP-43 have been directly linked to ALS. The aim of this study was to investigate whether mutant TDP-43 affects transport processes along the axons, which is important for the normal function of motor neurons. We used motor neurons derived from human induced pluripotent stem cell (hiPSC) as a model system. Fibroblasts from ALS patients with a N390S, a G287S or a A382T mutation in TARDBP were reprogrammed into hiPSC using Sendai virus-mediated expression of embryonic stem cell specific genes as well as by adding embryonic stem cell defining factors. Pluripotency of the obtained hiPSC was confirmed by quantitative PCR (qPCR) and immunohistochemistry (IHC). The hiPSC lines were subsequently differentiated into motor neurons, using a protocol provided by Dr. D. Bohl (Institut du Cerveau et de la Moelle épinière (ICM), France). The purity of the motor neuron cultures was confirmed by the expression of specific mature motor neuron markers, including Hb9, Isl1, ChAT, Smi32 and synapsin using IHC and qPCR. To study axonal transport, we labelled mitochondria in motor neurons with MitoTracker-RED. Subsequently, mitochondrial movement along the processes of the motor neurons was registered by live cell imaging, and the number of stationary and moving mitochondria was determined. Compared to control lines, the average number of moving mitochondria was significantly lower in motor neurons derived from patients with a TARDBP mutation. Our results clearly show that mutations in TARDBP cause impairments in axonal mitochondrial transport in hiPSC-derived motor neurons. This defect could eventually lead to the dysfunction and degeneration of the motor neurons.

**P78**

## ALS patient-derived astrocytes: a high throughput model for drug screening

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Rationale and hypothesis: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, incurable and fatal late onset neurodegenerative disease. Whilst the loss of motor neurons ultimately causes ALS pathology, astrocytes are strongly implicated in ALS disease progression. Riluzole is currently the only approved therapeutic for the disease, but only prolongs life by around 3 months. Therefore, therapeutics that are more efficacious are needed. New bioinformatics approaches using artificial intelligence to identify therapeutic targets have recently been developed. We are currently working with a biotech company, BenevolentAI Bio Ltd, to identify ALS therapeutics using this pioneer technology.
Objectives: To develop a pathophysiologically relevant, high-throughput screening assay to identify small molecules that alleviate astrocyte toxicity in ALS and validate a target-driven approach to drug discovery.
Methodology: Here, we have used ALS patient-derived skin fibroblasts, and converted them rapidly and directly to induced neuronal progenitor cells (iNPCs), which were then differentiated into iAstrocytes. These iAstrocytes were cultured in 384-well plates, and small molecules were delivered rapidly using an Echo550 liquid handler. Murine Embryonic Stem Cells (mESCs), engineered to contain a GFP gene under the control of the motor neuronal promoter, Hb9, were differentiated to produce Hb9-GFP+ murine motor neurons, which were then seeded in co-culture with the pre-treated human iAstrocytes. Hb9-GFP+ motor neurons were then imaged after 24 and 72 hours using an INCELL analyser 2000, and the number of viable motor neurons was counted using the Columbus™ analysis software.
Findings: ALS patient-derived iAstrocytes are toxic toward co-cultured Hb9-GFP+ murine motor neurons compared to healthy, control-derived iAstrocytes, which models astrocyte toxicity in ALS in vitro. Also, we have scaled up the co-culture assay for high-throughput screening of small molecules. Following an initial screening of few candidate compounds provided by our industrial collaborator, BenevolentAI Bio Ltd, we identified a promising hit that results in robust motor neuron rescue in our co-culture model. This lead compound inhibits several kinases, hence, we have performed a secondary screen in the co-culture model, and have identified four more specific kinase inhibitors that also provide a rescue effect, thus narrowing down the mode of action of the lead compound.

**P80**

## Novel combinatorial screening identifies neurotrophic factors for selective classes of ALS-relevant motor neurons

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Neurotrophic factors promote the survival of motor neurons but their combinatorial actions during normal development or in disease remain poorly understood. To address this, we screened 66 combinations of 12 neurotrophic factors on pure, highly viable and standardized embryonic mouse motor neurons isolated by a novel FACS technique. We demonstrate potent, strictly additive, survival effects of hepatocyte growth factor (HGF), ciliary neurotrophic factor (CNTF) and artemin through specific activation of their receptor complexes in distinct subsets of lumbar motor neurons: HGF supports hindlimb motor neurons through c-Met, CNTF supports subsets of axial motor neurons through CNTFRα, and artemin acts as the first survival factor for parasympathetic preganglionic motor neurons through GFRalphα3/Syndecan-3 activation. These data show that neurotrophic factors can selectively promote the survival of distinct classes of embryonic motor neurons. Similar data are under now gathered for postnatal motor neurons. Taken together, these studies provide a conceptual framework for the combined therapeutic use of neurotrophic factors in degenerative motor neuron diseases such as ALS, SMA and SBMA.

**P82**

## Macrophage migration inhibitory factor as a potential therapeutic candidate for familial ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease affecting both upper and lower motor neurons. The reason for the degeneration of motor neurons in ALS is still unknown. Intracellular organelles are suspected as a possible target for the misfolded SOD1 toxicity, not only in familial ALS cases with SOD1 mutations, but also in sporadic cases. The reason for why misfolded SOD1 specifically accumulates within motor neurons in ALS is still not fully understood. Recently, our laboratory succeeded to shed some light on this subject. A cytosolic factor which prevents the accumulation of misfolded SOD1 in unaffected tissues was identified as the 12 kDa macrophage migration inhibitory factor (MIF), a multifunctional protein that also possess a chaperone-like activity. Recombinant MIF inhibits misfolded SOD1 association with the mitochondria and ER membranes. In order to test the role of MIF in modulating SOD1 misfolding in vivo, MIF deficient mice were bred to mice expressing mutant SOD1G85R. Completely elimination of endogenous MIF accelerated disease onset and late disease progression and shortened lifespan of SOD1 mice. Higher amounts of misfolded SOD1 were detected in SOD1G85R-MIF-/- mice through disease course within the spinal cord, brain and liver compared to the SOD1G85R littermates. In addition, misfolded SOD1 association with mitochondria and ER membranes was significantly higher in the spinal cord of SOD1G85R-MIF-/- compared to their SOD1G85R mice. On the other hand, we are using adeno associated viral (AAV) vectors to overexpress MIF in the CNS of mutant SOD1 mice to determine whether upregulation of MIF can slow down the disease course. From our preliminary results, MIF accumulation levels were increased in the spinal cords and brains of the injected mutant SOD1 mice. Our findings indicate that MIF plays a significant role in SOD1 folding and misfolding mechanisms in vivo. These results strengthen the hypothesis that MIF acts as a chaperone for misfolded SOD1 and they have implications regarding the therapeutic potential role of upregulation of MIF in modulating the specific accumulation of misfolded SOD1.

**P84**

## Muscle microRNAs: smallRNA sequencing and differential expression in Slovenian patients and healthy age-matched controls

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MicroRNA (miRNAs) are short non-coding RNAs that serve as important regulators of gene expression and can act both globally and in a tissue- or cell-type specific manner. Muscle miRNAs are involved in various processes, such as myogenesis, muscle homeostasis, response to exercise, as well as muscle atrophy due to aging, immobility and muscular and neuro-muscular disorders. As specific regulators, in animal and cellular models, muscle miRNAs have shown promise for therapeutic use. Our study aimed to identify, for the first time, miRNAs differentially expressed in muscle biopsy tissue of ALS patients vs. healthy age-matched controls, by using next generation small RNA sequencing (small RNA Seq) and bioinformatics analysis. In total 11 ALS patients and 11 controls were included in the study. Analysis of small RNA Seq showed approx. 30 microRNA families/species to be differentially expressed between the patient and control groups. Bioinformatics revealed the differentially expressed microRNA have several thousand potential protein targets, with some of the proteins being targeted by multiple miRNAs. Of the targets, approx. 40 have already been implicated in ALS pathology in neurological tissue or other known disorders involving muscle wasting, while several hundred other targets are known to be involved in muscle contraction, muscle organ development, skeletal muscle cell differentiation, muscle morphogenesis etc. The study represents an important first step in determining possible novel target approaches in slowing or stopping atrophy of muscle tissue in ALS.

**P86**

## TBI causes a transient elevation of pTDP-43-positive cytoplasmic granules in ALS-relevant mouse models

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Traumatic brain injury (TBI) has been proposed as a risk factor for neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). To determine whether TBI might exacerbate ALS-relevant injuries, we performed a highly reproducible mild stab-wound injury in the motor cortex of three different mouse models expressing mutations in SOD1, TDP-43 or FUS, relevant to familial cases of ALS. We analyzed the effects of TBI on TDP-43 localisation in cortical neurons, as well as on survival and motor phenotype of transgenic mice. Stab-wound TBI caused an elevation of pTDP-43-positive cytoplasmic granules in wild type animals, and this was exacerbated upon expression of TDP-43G298S or of truncated FUSΔNLS. Indeed both mutant TDP-43 and mutant FUS transgenic mice displayed a higher load of cytoplasmic pTDP-43 granules that peaked 7 days after injury and returned to baseline 90 days after injury. Intriguingly, the expression SOD1G93A abolished the accumulation of pTDP-43 cytoplasmic granules. Despite the prominent increase in cytoplasmic pTDP-43 granules, no additional neuronal loss was detected in all ALS mouse models analyzed and survival and overall motor behavior were not affected by motor cortex injury. In summary, our data demonstrate that genetic mutations relevant to ALS exacerbate the pathological effects of a mild single stab wound. Repetition of TBI could thus influence the development of typical ALS lesions.

**P88**

## The fecal microbiome of early-stage ALS patients

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ALS is a fatal neurodegenerative motor neuron disease accompanied by both systemic and CNS-specific inflammation as well as deregulated energy metabolism. All these processes have recently been found to be influenced by the gut microbiota, raising the hypothesis of a link between microbiome alterations and ALS pathogenesis. Limited evidence for an altered microbiota in ALS recently was provided by a small, preliminary trial with 6 patients and studies in the SOD1-G93A mouse model. The aim of this pilot study was to assess whether ALS is associated with an altered composition of the fecal microbiota. We compared the fecal microbiota of 25 early-stage ALS patients with 32 age- and gender-matched healthy persons using 16S rRNA gene sequencing analysis. Confounding factors and secondary disease effects on the microbiome were excluded by selection of early-stage patients without dysphagia, gastrostomy, non-invasive ventilation, reduced body mass index or long disease course. Comparing the two carefully matched groups, the quantity, the diversity and the abundance of the bacterial taxa on the different taxonomic levels as well as PICRUSt predicted metagenomes were not significantly different. Conclusively, early stage ALS patients do not exhibit an alteration of the gut microbiome composition, which is therefore most likely not associated with the causation of this disease.

**P90**

## Modeling FUS-ALS hallmark neuropathology using patient-specific iPSCs and iPSC-derived cortical neurons

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Amyotrophic lateral sclerosis (ALS) is an adult onset disorder in which about 5% of familial cases are caused by autosomal-dominant mutations within the FUS (fused in sarcoma) gene. ALS is considered as aggregate prone disease with spreading of disease pathology during disease progression, affecting spinal and cortical motor neurons. We use patient-specific and isogenic induced pluripotent stem cell (iPSC) lines to model FUS-ALS in human in vitro cultures. That allows the (patho-)physiological investigation of FUS in iPSCs and iPSC-derived cortical neurons carrying endogenous mutation. We analyze iPSC-derived cortical neurons during different steps of cortical layer differentiation and maturation and compare these to hindbrain/spinal neuronal phenotypes. We found typical hallmarks of neuropathology including aggregate formation, cytoplasmic mislocalization and neurodegeneration. We found that the amount of cytoplasmic FUS depends on the severity of the underlying mutation. Cytoplasmic FUS inclusions formed spontaneously in mutated iPSC-derived cortical neurons but not iPSCs depending both on the severity of FUS mutation and neural aging. Our study thereby highlights the value and usefulness of patient-derived cell models in FUS-ALS and the importance to study pathophysiology in cell types specifically affected in disease.
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**P92**

## Dynamics of AGO2 interactions with nuclear ALS proteins in stressed neurons

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease in which motor neurons (MNs) are degenerated leading to muscle atrophy, paralysis and eventually death. Several lines of evidence link the pathophysiology of ALS to dysregulation of microRNAs (miRNAs), a subset of small non-coding RNAs implicated in post-transcriptional regulation of gene expression. miRNAs are essential to almost all aspects of neuronal development and function, and their dysfunction has been implicated in several other neuro-pathological conditions as well. Argonaute 2 (AGO2) is the core effector protein of miRNAs, enabling sequence-based silencing capacity. Although AGO2 is primarily found in the cytoplasm, recent data demonstrate novel functions of AGO2 in the nucleus, contributing to transcriptional regulation, alternative splicing and DNA repair. We explored the landscape of nuclear AGO2-interacting proteins in neurons, under normal or stress conditions, which simulate diseased neurons, by unbiased mass-spectrometric analysis. Nuclear AGO2 interacted with ~30 proteins, many of which are associated with RNA-processing. Following stress induction, AGO2 interactions were altered; thus, while the interaction of AGO2 with nuclear paraspeckle components remained unchanged under stress conditions, new interactions of AGO2 with TDP-43, FMRP and PFN1 emerged. Importantly, the three stress-dependent interactors are proteins that are implicated in neurodegenerative diseases. Specifically, TDP-43 and PFN1 protein mutations are sufficient to cause ALS. Our data reveals a compound and dynamic network of AGO2 interactions that are sensitive to stress and encourage future investigations on the functional relevance of these interactions to neuronal function and survival.

P94

## ALS, gene deregulation in the anterior horn of the spinal cord and frontal cortex area 8: implications in frontotemporal lobar degeneration

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Transcriptome arrays identifies 747 genes differentially expressed in the anterior horn of the spinal cord and 2,300 genes differentially expressed in frontal cortex area 8 in a single group of typical sALS cases without frontotemporal dementia compared with age-matched controls. Main up-regulated clusters in the anterior horn are related to inflammation and apoptosis; down-regulated clusters are linked to axoneme structures and protein synthesis. In contrast, up-regulated gene clusters in frontal cortex area 8 involve neurotransmission, synaptic proteins and vesicle trafficking, whereas main down-regulated genes cluster into oligodendrocyte function and myelin-related proteins. RT-qPCR validates the expression of 58 of 66 assessed genes from different clusters.
The present results: a. reveal regional differences in de-regulated gene expression between the anterior horn of the spinal cord and frontal cortex area 8 in the same individuals suffering from sALS; b. validate and extend our knowledge about the complexity of the inflammatory response in the anterior horn of the spinal cord; and c. identify for the first time extensive gene up regulation of neurotransmission and synaptic-related genes, together with significant down-regulation of oligodendrocyte- and myelin-related genes, as important contributors to the pathogenesis of frontal cortex alterations in the sALS/frontotemporal lobar degeneration spectrum complex at stages with no apparent cognitive impairment.

P96

## Generation of gene knockouts and gene replacements in human cell lines for disease modelling: a cautionary note

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The development of CRISPR/Cas9 offers the possibility to knockout almost any gene of interest in an affordable and simple manner. The generation of a gene knockout usually relies on the introduction of a frameshift into the open reading frame of the target gene which truncates the coding sequence and targets the corresponding transcript for degradation by the nonsense-mediated mRNA decay (NMD) pathway. We show that transcripts containing premature termination codons are often degraded inefficiently and these transcripts can generate C-terminally truncated proteins which might have residual or dominant negative functions. Therefore, we propose an alternative approach to knockout genes which completely prevents the expression of the target gene avoiding C-terminally truncated protein expression and prove its feasibility for targeting several genes (FUS, SMG7) and different human cell lines. Additionally, we show targeting TARDBP, that this approach can be used to efficiently generate gene replacements.

**P98**

## MIF inhibits the formation of misfolded SOD1 amyloid aggregates: implications for familial ALS

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Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS) – a progressive and fatal neurodegenerative disease characterized by the loss of upper and lower motor neurons in the brain and spinal cord. It has been suggested that the toxicity of mutant SOD1 in ALS results from its misfolding, but, to date, it is yet unclear why misfolded SOD1 accumulates specifically within motor neurons. We recently demonstrated that the macrophage migration inhibitory factor (MIF)—a cytosolic multifunctional protein with a chaperone-like activity—inhibits the accumulation of misfolded SOD1 in ALS-unaffected tissues. In the current study, we investigated the mechanism underlying this protective function and show that MIF alters the typical amyloid aggregation pathway of misfolded SOD1 and, instead, promotes the formation of disordered aggregates with lower toxicity. Moreover, we report that MIF reduces the toxicity of misfolded SOD1 by directly interacting with it, and that the chaperone-like function and protective effect of MIF do not require its known enzymatic activities. Altogether, our study provides mechanistic insights into the ability of MIF to modulate the specific accumulation of misfolded SOD1 which may have therapeutic implications for ALS.

**P100**

## Understanding mechanisms of truncating mutations in the FUS gene

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A number of mutations in the FUS gene can cause familial ALS (ALS-FUS). Mutant FUS protein is prone to mislocalise to the cytoplasm and form cytoplasmic aggregates as well as be recruited to stress granules under stress conditions. Mutations most commonly affect the C-terminal part of FUS protein, and the majority of them are missense mutations in the gene region encoding FUS nuclear localisation signal (NLS). However currently more than ten frameshift and splice acceptor site mutations are known which lead to complete absence of NLS and production of a truncated protein; such mutations are often associated with early disease onset. Mechanisms of toxic gain of function conferred by such mutations may be different from those triggered by missense mutations since the protein's structure is more profoundly affected in the former case. Precise modelling of modifications in the FUS protein arising due to frameshift/splice acceptor site mutations has not been performed so far. To fill this gap in our knowledge, in current study we use both transient expression and CRISPR/Cas9-mediated gene editing to model changes in the distribution and function of FUS protein caused by such mutations. In particular, precise gene editing has been employed to reproduce exact changes in FUS protein structure due to splicing site mutations. The effect of these modifications on stress granule dynamics, aggregation and toxicity has been assessed. Our study provides insights into previously unrecognised pathological mechanisms in a subset of ALS-FUS cases.

**PI02**

## Sensory neuropathy in progressive motor neuronopathy (pmn) mice is associated with defects in microtubule polymerization and axonal transport

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Motor neuron diseases such as ALS are now recognized as multi-system disorders also involving various non-motor neuronal cell types. The precise extent and mechanistic basis of non-motor neuron damage in human ALS and ALS animal models remain however unclear. To address this, we here studied pmn (progressive motor neuronopathy) mice carrying a missense loss-of-function mutation in TBCE (tubulin binding co-factor E). These mice manifest a particularly aggressive form of motor axon dying back and display a microtubule loss, similar to that induced by human ALS-linked TUBA4A mutations. Using whole nerve confocal imaging of pmn x thy1.2-YFP16 fluorescent reporter mice and electron microscopy, we demonstrate axonal discontinuities, bead-like spheroids and ovoids in pmn suralis nerves indicating prominent sensory neuropathy. The axonal alterations qualitatively resemble those in phrenic motor nerves but do not culminate in the loss of myelinated fibers. We further show that the pmn mutation decreases the level of TBCE, impedes microtubule polymerization in dorsal root ganglion (DRG) neurons and causes progressive loss of microtubules in large and small caliber suralis axons. Live imaging of axonal transport using GFP-tagged tetanus toxin C-fragment (GFP-TTC) demonstrates defects in microtubule-based transport in pmn DRG neurons, providing a potential explanation for the axonal alterations in sensory nerves. This study unravels sensory neuropathy as a pathological feature of mouse progressive motor neuronopathy, and discusses the potential contribution of cytoskeletal defects to sensory neuropathy in human motor neuron disease.

**PI04**

## Interactions between TAU, FUS and TDP-43 in neurodegenerative diseases

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Three mutually exclusive neuropathological inclusions are found in ALS/FTD patients: cytoplasmic inclusions of TDP-43 (TDP-43 proteinopathy), FUS (FUSopathy) or TAU proteins (so-called TAUopathy). TDP-43 and FUS are RNA-binding proteins involved in multiple steps of RNA metabolism, from transcription to alternative splicing and transport. TAU protein is a cytoskeletal protein that is critical for the stability of microtubule structure and functions in neurons. Further strengthening the link between these three proteins and ALS/FTD, mutations in TDP-43 and FUS genes have been identified in ALS and mutations in TAU are associated with a significant proportion of familial FTD. In all, pathology and genetics converge to ascribe a predominant pathogenic role for TDP-43, TAU and FUS in the development of ALS/FTD. Defining the mechanistic relationships between TDP-43, FUS and TAU is the subject of the current application. We will focus our work on the TAU/FUS epistatic interaction in zebrafish models and our major objective is to evaluate the contribution of FUS to TAUopathies, and of TAU to FUSopathies. In parallel, we will explore possible interactions between TAU and TDP-43. For this, we plan to co-express mutant and WT TAU alongside mutant FUS or WT FUS and/or mutant TDP-43 or WT TDP-43 and score for phenotypic features, including swimming trajectories following escape response test and axonal projections from spinal motor neurons. We also intend to develop and characterize deletion mutant lines of FUS and TDP-43 using CRISPR/Cas9 technology and to determine phenotypic features associated with loss of function of these factors. Also, we will cross these transgenic animals with mutant TAU transgenic zebrafish lines. Finally, bioactive compounds will be tested in several of these models described above to determine the neuroprotective properties of these drugs and to define novel therapeutic strategies for ALS and FTD. We expect to confirm genetic interactions between FUS and TAU using epistatic analysis in order to comprehend whether FUS is upstream or downstream of TAU. The role of mutant TDP-43 will also be mapped in this genetic interaction crucial for our understanding of the pathogenic mechanisms in ALS-FTD.

**P106**

## Profiling of basement membrane proteins in plasma and cerebrospinal fluid within ALS and frontotemporal dementia

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Muscle weakness and atrophy as a result of motor neuron degradation are the primary and most prominent symptoms of amyotrophic lateral sclerosis (ALS). Several recent studies however, also indicate that a vascular disease component is present among the ALS symptoms. Both the blood-brain barrier and the blood-spinal cord barrier are damaged in patients with ALS and transgenic mouse models show signs of disruption prior to disease onset. To investigate the connection to vascular disruption we profiled about one hundred basement membrane proteins in ALS patient plasma. By directly labeling the samples with biotin and coupling antibodies to magnetic color-coded beads, more than 1 200 samples from Belgium, Germany, the Netherlands, Poland, Sweden and the US. Both patients and healthy controls were profiled and relative protein amounts were detected in a flow cytometry-like system by addition of a streptavidin-coupled fluorophore. The clinical data connected to the samples will enable us to study protein levels in association to age at onset, disease duration and progression rate. We have further extended our study to profile the same proteins in cerebrospinal fluid from a smaller set of frontotemporal dementia (FTD) patients. Although ALS and FTD are heterogeneous at the clinical and neuropathological level they share several features, not least abnormal aggregation of the TDP-43 protein. By including FTD patients in our study we wish to a) evaluate if the measured proteins could have a potential relevance for FTD and b) attempt to determine if the observed protein patterns are ALS-specific or overlapping between the two diseases. Our overall aim is to better understand if and how basement membrane disruption contributes to ALS pathology, and map the potential protein overlap with FTD.

**PI08**

## Progranulin functions as a cathepsin D chaperone to stimulate axonal outgrowth in vivo

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Loss of function mutations in progranulin (GRN) cause frontotemporal dementia, but the mechanism of neuronal dysfunction remains unclear. We previously showed that GRN is neurotrophic in vitro. Here, we used an in vivo axonal outgrowth system and observed a delayed recovery in GRN-/- mice after facial nerve injury. This deficit was rescued by reintroduction of human GRN and completely relied on its C-terminus and neuronal GRN production. Transcriptome analysis of the facial motor nucleus post injury identified cathepsin D (CTSD) as the culprit. In aged GRN-/- cortices, relative CTSD activity was reduced and improved upon exogenous GRN addition. Moreover, GRN and its C-terminal granulin domain granulinE (GrnE) both stimulated the proteolytic activity of CTSD in vitro. Pull-down experiments confirmed a direct interaction between GRN and CTSD. This interaction was also observed with GrnE and stabilized the CTSD enzyme at different temperatures. Investigating the importance of this interaction for axonal regeneration in vivo, we found that although individual tolerated, a combined reduction of GRN and CTSD synergistically reduced axonal outgrowth. Overall, our data links the neurotrophic effect of GRN and GrnE with a lysosomal chaperone function on CTSD to maintain its proteolytic capacity.

**P110**

## Unravelling the molecular mechanisms behind corticospinal motor neuron degeneration in ALS

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Amyotrophic lateral sclerosis (ALS) is characterized by the combined degeneration of the corticospinal motor neurons (CSMN) and the spinal motor neurons (SMN). Despite this description, the signalling pathways behind CSMN degeneration and the pathology that develops within the surrounding cerebral cortex during the course of the disease are still largely unknown. This project aims at better characterizing the cortical pathology that characterizes ALS, and at deciphering the molecular mechanisms that selectively trigger CSMN degeneration. In the Sod1G86R mouse model of the disease, we first tested whether CSMN degenerate. We quantified the number of layer V Crym-positive neurons present in the motor areas, and counted the number of CSMN retrogradelly labelled from the cervical or lumbar parts of the spinal cord. Both approaches showed that CSMN progressively and significantly degenerate in Sod1G86R mice over time. In addition, we observed that the subpopulation of CSMN that project to the lumbar spinal cord, where motor neurons that innervate the hindlimbs are located, degenerate earlier and to a greater extend than the broad CSMN population. Given that in this mouse model motor symptoms appear first in the hindlimbs, the data show that Sod1G86R recapitulate a progressive degeneration of the CSMN in a somatotopic manner, as reported in the patients. To better characterize the cortical pathology, we performed a first series of molecular and histological analyses on the motor cortex of the Sod1G86R mice. Our preliminary results show that, as opposed to the well-characterized spinal pathology, the cortical pathology occurs in absence of major gliosis. Finally, to shed light on the molecular mechanisms that selectively trigger CSMN degeneration during the course of ALS, we developed a method to purify CSMN from the cerebral cortex of adult wild type and Sod1G86R mice at pre-symptomatic and symptomatic ages, in order to perform a temporal RNAseq analysis. We are currently mining these RNAseq data and validating a first series of candidate genes. On the long run, this project aims at identifying new signalling pathways that may in turn inform the development of alternative therapeutic strategies for ALS.

**PII2**

## Generation and analysis of ALS-associated hnRnp A knockout zebrafish lines

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease for which currently no effective therapies are available, mainly due to the limited understanding of the disease-causing molecular mechanisms. Increasing evidence points to a central role of RNA-binding proteins and impaired RNA metabolism in ALS. The majority of genetic mutations associated with familial ALS are found in genes with RNA-binding function and the proteins encoded by these genes were shown to impact directly on RNA processes, such as gene transcription, pre-mRNA splicing, RNA translation or degradation. This evident overrepresentation of RNA-binding proteins highlights their importance for disease mechanisms in ALS. In this study we focus on the RNA-binding proteins hnRNP A1, hnRNP A2B1 and hnRNP A3. Mutations in hnRNP A1 and hnRNP A2B1 were found in ALS patients and hnRNP A3 was shown to form intranuclear inclusions in patients with C9orf72 repeat extensions. Although these proteins have numerous functions in RNA processing, their role in the central nervous system is poorly understood. To determine the physiological function of hnRnp As and to test whether loss of hnRnp As is necessary and sufficient to elicit ALS related pathology, I generated zebrafish knockout lines of these three genes by CRISPR/Cas9 genome editing. Knockout lines for hnRnp A1, hnRnp A2, and hnRnp A3 interestingly did not show obvious phenotypic consequences, as they were fertile and viable, indicating that pathogenicity of one absent hnRnp A protein is not sufficient to elicit ALS reminiscent symptoms and pathology in zebrafish. Next, I will generate double and triple KO lines of the hnRnp As to uncover potential functional redundancy. To determine potential changes in RNA levels of other proteins, I will perform RNAsequencing and confirm identified hits by quantitative real time PCR (qRT-PCR). Combined, from these findings we will better understand hnRnp A function in vivo and the extensive cross-talk between hnRnP As, which will allow us to reveal potential downstream molecular convergence and is expected to contribute in the understanding of ALS pathology.

**PII4**

## Mitochondrial Calcium Uniporter expression with disease progression in the G93AhSOD1 mouse model

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Background: Abnormalities in the ER-mitochondria Ca2+ cycle, which lead to mitochondrial Ca2+ overload, are important in selective motor neuron death (1). Ca2+ is channelled through the inner mitochondrial membrane by the mitochondrial calcium uniporter complex (MCU) and through the outer mitochondrial membrane by the voltage dependent anion channel (VDAC). Our previous results in cultured embryonic G93AhSOD1 motor neurons showed altered MCU expression and reduction of mitochondrial Ca2+ buffering capacity. Moreover, MCU activation using kaempferol results in increased spontaneous Ca2+ activity in the presence of mutated hSOD1. MCU downregulation using a protein kinase type II (CAMKII) inhibitor, KN-62 seems to be protective against AMPAR-mediated excitotoxicity (2).
Objectives: As MCU seems to play an important role in mitochondrial Ca2+ overload, our objectives were to explore the MCU protein expression and mRNA levels in G93AhSOD1 mice during disease progression.
Materials and Methods: Cervical section of spinal cords from G93AhSOD1 mice prior to (8-9 weeks) and after the onset of disease-related symptoms (19-21 weeks) were used for the experiments. Since they were bred on an identical background, age matched C57BL/6J mice were used as controls. MCU expression was analyzed by immunocytochemistry using corrected total cell fluorescence in FIJI. RT-qPCR was done to investigate mRNA level.
Hypothesis: It is assumed that mitochondrial Ca2+ handling is remodeled during ALS progression from mitochondrial uptake to mitochondrial uptake failure and increased plasma membrane extrusion (3). Our longitudinal study highlights the role of MCU expression during the disease progression and will provide better understating of mitochondrial Ca2+ buffering and its adoption under pathophysiological conditions.
Acknowledgment: This research is supported by BMBF (Bundesministerium für Bildung and Forschung) in the framework of the E-RARE programme (PYRAMID) and JPND (OnWebDUALS).
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**P116**

## Clinical, pathological and molecular characterisation of C9orf72-ALS leads to identification of novel therapeutic targets

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My aim has been characterisation of the recently discovered genetic variant of amyotrophic lateral sclerosis (ALS) associated with G42C-repeat expansion of C9orf72. My research has largely utilised patient tissue and biosamples in order to retain focus on the human disease. First we described the broad clinical phenotype of C9orf72-ALS (Cooper-Knock et al. 2012). Recognising that extramotor presentations are the hallmark of C9orf72-ALS, I examined the role of C9orf72-expansions in both parkinsonism (Cooper-Knock et al. 2013) and multiple sclerosis (Ismail et al. 2013). This led to clinically relevant insights, including enhancement of C9orf72 penetrance by concurrent multiple sclerosis, the presence of substantia nigra pathology in C9orf72-ALS cases, and a C9orf72-specific downregulation in CSF levels of the neuroprotective cytokine CXCL10. My work using immunohistochemistry to characterise molecular phenotypes in C9orf72-patient post-mortem tissue has been significantly impactful in the field. We identified pathognomonic extra-motor pathology (Cooper-Knock et al. 2012) and described interactions between RNA-repeat molecules and RNA-binding proteins in CNS tissue and in vitro (Cooper-Knock et al. 2014). We were the first to detail variation in frequency of RNA foci between neuronal populations (Cooper-Knock et al. 2015). Our discovery of a correlation between antisense transcription and motor neuron pathology (Cooper-Knock et al. 2015) was entirely novel but has been supported by later work in mouse models (Liu et al. 2016) and more recently in iPS-derived motor neurons (Liu et al. 2017); antisense transcripts will be an essential target of antisense oligonucleotides in future therapeutic trials. Next I chose to examine the lengths of C9orf72 expansions. I performed Southern blots on a large number of C9orf72-patient biosamples including peripheral blood, lymphoblastoid cells, saliva and CNS tissue (Buchman et al. 2013). This enabled us to demonstrate somatic heterogeneity in the repeat length (Buchman et al. 2013) and we were among the first to determine the effect of repeat length on C9orf72 mRNA transcription (Cooper-Knock et al. 2013). Finally, using an entirely novel transcriptome analysis, we described a quantitative correlation between RNA splicing consistency and disease severity (Cooper-Knock et al. 2015).

**P118**

## TDP-43-nucleoporin connection: a novel player in ALS?

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In this work we addressed if the protein subunits that build the nuclear pore, nucleoporins (NUPs) are altered as an underlying mechanism in the etiopathogenesis of ALS. We examined, using TDP-43 as paradigm, whether NUPs dearrangement could contribute to cytosol protein offshoring and its potential aggregation. To do this, we analysed the levels of several NUPs by immunodetection techniques in isolated tissues and nuclei extracted post mortem from ALS patients, as well as in a transgenic murine model of ALS in several stages of the disease and in both genders. In addition, we performed cell culture studies to elucidate the possible mechanisms that influence NUPs-mediated TDP-43 dysregulation. In this context, the relationship between cell stress, TDP-43 and NUPs was discussed to establish the possible influence of stress mechanisms on the distribution of specific NUPs and their relation to the TDP-43 pathological characteristics. The results demonstrate changes in the levels of NUPs involved in the recognition of transporter proteins in both post-mortem tissues from ALS patients and in model mice from the disease. On the other hand, the silencing of one of the NUPs, NUP107, caused an increase in the levels of TDP-43 and its phosphorylation, as well as an increase in the formation of its cytoplasmic aggregates. In addition, this was associated with autophagic response alterations, evidenced by the increase of LC3II, p62 and the levels of general protein ubiquitination. Similarly, oxidative stress and osmotic stress in vitro, caused an increase in the pathological characteristics of TDP-43 mentioned above, an increase associated with changes in the expression of NUPs. These findings demonstrate that the deterioration of NUPs in the ALS framework may be a contributing mechanism to the alteration of intracellular traffic resulting in the proteins aggregation involved in motoneuronal neurodegeneration, such as TDP-43.

**PI20**

## Oral supplementation with the omega-3 docosahexaenoic acid (DHA) in patients with amyotrophic lateral sclerosis (ALS): a randomized, double-blind, placebo-controlled pilot study

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Rationale: Patients with amyotrophic lateral sclerosis (ALS) have progressive muscle atrophy with high levels of circulating inflammatory markers that express the body's inflammatory state and oxidative stress. There are increasing solid evidences of the important role of a correct dietary intake of omega-3 fatty acids, such as DHA. Their anti-inflammatory activity, as well as, their contribution to the normal function of the nervous system is expected to have a positive effect on ALS patients.
Methods: To evaluate the possible benefits of DHA on ALS patients, an oral supplementation of 1g of DHA (+ vitamin E) was given to a group of 14 patients, while another similar group received 1g of olive oil as a placebo. The supplementation was for one year. Blood samples of two groups were collected every 3 months to evaluate levels of cholesterol and inflammatory biomarkers as interleukins. Besides that, weight loss pre-diagnosis, ALS Functional Rate Scale (ALSFRS) and forced vital capacity (FVC) as clinical parameters were recorded.
Results: At baseline time 28 ALS patients were included but only 16 completed the follow up. No significative differences were found on weight loss pre-diagnosis, diagnostic delay, gender, onset site and age.
Conclusion: From the results, a clinical improvement in the treated group cannot be concluded due to the small size of the sample (limitation of the study). TNF alfa levels have decreased in the treated group but the differences are weakly significatives. Further studies with an increased number of ALS patients would be necessary to confirm this anti-inflammatory activity.

**PI22**

## A safety analysis of edaravone (MCI-186) during the first 6-cycles (24 weeks) of ALS therapy from 3 randomized double-blind placebo-controlled trials

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Background: The safety analysis reported herein was based on 3 randomized placebo-controlled clinical trials (RCTs) [MCI186-16, MCI186-18, and MCI186-19]. Two other studies, an open-label phase II study (MCI186-12) and an extension study of MCI186-16 (MCI186-17), were excluded from the analysis.
Objectives: Provide an overview of safety for edaravone (60 mg intravenous once-daily) in ALS subjects during the first 6 cycles (24 weeks) of treatment.
Methods: Safety data from the double-blind period (the first 6-cycles of treatment) in the 3 phase III RCTs were pooled for this analysis. The safety endpoints included, but were not limited to, treatment-emergent adverse events (TEAEs) including deaths, serious adverse events (SAEs), and AEs leading to discontinuation.
Results: The analysis included a total of 368 patients (184 each in the edaravone [E] group and placebo [P] groups). Of those, 94.6% of E and 90.2% of P completed 6 cycles of therapy. Baseline demographics and disease characteristics were comparable between the 2 groups. TEAE incidence in E and P was 87.5% and 87%, respectively. The most frequently reported severe TEAEs in either group were gait disturbance [5.4% (E) and 2.7% (P)]; dysphagia [3.3% (E) and 4.9% (P)]; and musculoskeletal disorder [2.2% (E) and 2.7% (P)]. The incidence of treatment-emergent SAEs was 17.4% in E and 22.3% in P. There were no investigator reported drug-related SAEs in either group. There was no significant difference in SAEs by treatment cycle between the 2 groups. Treatment-emergent deaths occurred in 2.2% in E group and 1.1% in P group, all of which were respiratory in nature and attributed to worsening ALS. There was no imbalance observed of note in incidence of AEs leading to discontinuation between the 2 groups [2.2% (E) and 5.4% (P)].
Discussion: While some TEAEs were more common in the E group compared to P group, the incidence of SAEs, deaths, and discontinuations due to AEs were similar or less for E compared to P.
Conclusion: Data collected from 3 double-blind assessments of edaravone suggest an acceptable safety profile in patients with ALS.

**PI24**

## Pharmacokinetic profile of edaravone: a comparison between Japanese and Caucasian populations

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Background: Amyotrophic lateral sclerosis (ALS) affects persons of all races, and there continues to be a need for effective therapies to treat the disease.
Objective: We performed a population pharmacokinetic analysis (PPK) to compare the pharmacokinetics of edaravone in Japanese and Caucasian populations.
Methods: A total of 5 pharmacokinetic (PK) studies in healthy volunteers among Japanese (3 studies) and Caucasian (2 studies) populations were evaluated. The PPK model was constructed using non-linear mixed effect modeling. Covariate effects by race, gender, weight, and age were investigated to explain variability in the PK parameters, including maximum plasma concentration (Cmax, terminal plasma concentration (Ctau), and area under the plasma-concentration time curve (AUC). Simulations of the final PPK model, using a virtual population based on published literature of ALS clinical trials in Europe and the United States, were used to support dose extrapolation.
Results: The PPK analysis included 86 subjects, with a near-equal distribution of Japanese and Caucasian subjects, 54.7% vs 45.3%. Their mean age (SD) was 45.8 (17.4) years and 76.7% were male. A 3-compartment model with Michaelis-Menten plus linear elimination was selected as the best fit model. Race was statistically detected as a covariate for the second peripheral volume of distribution (V2), indicating a 26% increase for Caucasian subjects compared to Japanese subjects. The small difference of V2 was associated with a difference of Ctau around 1 ng/mL based on the virtual population. This difference was minimal compared to Cmax (approximately 1000 ng/mL), and did not result in the accumulation of drug concentration after multiple dosing. No significant differences were observed for Cmax or AUC between the populations. Gender, age, or weight did not affect any PK parameters.
Conclusion: The PPK analysis of edaravone demonstrated a 26% increase of V2 for Caucasian subjects compared to Japanese subjects, but the difference of V2 was minimal compared to Cmax and it did not result in the accumulation of drug concentration. There were no significant differences in Cmax or AUC between the populations. The PK parameters for edaravone were not affected by gender, weight, or age.

**PI26**

## A pharmacometabolomics approach in ALS: proof of concept in a clinical trial of olesoxime

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10. Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France

Background: As a proof of concept of pharmacometabolomics in amyotrophic lateral sclerosis (ALS), we analysed some plasma samples collected during the therapeutic trial of TRO19622 (olesoxime) in ALS. The main objective was to investigate the link between metabolome modifications, from the early beginning of the study, and late clinical outcomes.
Material and methods: Patients included in the trial received riluzole and were randomized to one of two groups: Group O receiving olesoxime (n=38) and Group P receiving placebo (n=36). The metabolome was assessed at one (V1) and 12 months (V12) after the initiation of the treatment. High performance liquid chromatography coupled with tandem mass spectrometry was used to quantify 188 metabolites (Biocrates® commercial kit). Multivariate analysis based on different learning machine methods (i.e. Biosigner algorithm) was performed.
Results: Metabolome profiles at V1 and V12 and variation of metabolomes between V1 and V12 correctly discriminated between Groups O and P ($p<5\times10^{-6}$), with glycine, kynurenine and citrulline/arginine as the most discriminant metabolites. Changes in metabolome profiles were closely linked with clinical progression, with a significant correlation between glutamine levels in Group P and amino acids, lipids and spermidine levels in Group O. Multivariate models correctly predicted disease progression from the V1 metabolome, including the discriminant role of sphingomyelins (SM C22:3 or SM C24:1) when pooling data from both groups.

Conclusion: This study provides proof of concept that the metabolome may be of use in assessing the biological effect of an investigational drug and of interest as a secondary outcome measure in clinical trials.

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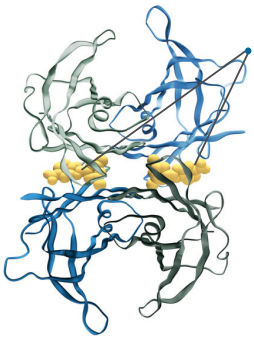

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**SESTAVA:** Ena kapsula Duloksetin Sandoz 30 mg trde gastrorezistentne kapsule vsebuje 30 mg duloksetina (v obliki duloksetinijevega klorida) ter 1,95 mg laktoze (v obliki laktoze monohidrata) in 0,167 mg barvila alurno rdeče AC (E129) kot pomožni snovi z znanim učinkom. Duloksetin Sandoz 60 mg trde gastrorezistentne kapsule vsebuje 60 mg duloksetina (v obliki duloksetinijevega klorida) ter 3,9 mg laktoze (v obliki laktoze monohidrata), 0,152 mg barvila sončno rumeno FCF (E110) in 0,390 mg barvila alurno rdeče AC (E129) kot pomožne snovi z znanim učinkom. Za celoten seznam pomožnih snovi glejte poglavje 6.1. SmPC-ja. **TERAPEVTSKE INDIKACIJE:** Zdravljenje velikih depresivnih motenj. Zdravljenje bolečine diabetične periferne nevropatije. Zdravljenje generaliziranе anksiozne motnje. Zdravilo Duloksetin Sandoz je indicirano za zdravljenje odraslih. **ODMERJANJE IN NAČIN UPORABE:** Odmerjanje: *Velike depresivne motnje:* Začetni in priporočeni vzdrževalni odmerek je 60 mg enkrat na dan s hrano ali brez nje. V kliničnih preskušanjih so z vidika varnosti vrednotili odmerjanja prek 60 mg enkrat na dan, do najvišjega odmerka 120 mg na dan. Vendar pa ni kliničnih dokazov, ki bi kazali, da lahko bolnikom, ki se na priporočeni začetni odmerek ne odzovejo, povišanje odmerka koristi. Terapevtski odziv običajno opazimo po 2–4 tednih zdravljenja. Po ustaljenem odgovoru na antidepresivno zdravilo je priporočljivo nadaljevati zdravljenje še nekaj mesecev, da se izognemo relapsu. Pri bolnikih, ki se odzovejo na zdravljenje z duloksetinom in imajo v anamnezi ponavljajoče epizode velikih depresij, lahko razmislimo o nadaljnjem dolgotrajnem zdravljenju z odmerkom od 60 do 120 mg na dan. *Generalizirana anksiozna motnja:* Priporočeni začetni odmerek pri bolnikih z generaliziranо anksiozno motnjo je 30 mg enkrat na dan s hrano ali brez nje. Pri bolnikih z nezadostnim odgovorom moramo odmerek zvišati na 60 mg, kar je običajen vzdrževalni odmerek pri večini bolnikov. Pri bolnikih s sočasnimi veliki depresivnimi motnjami je začetni in vzdrževalni odmerek 60 mg enkrat na dan (prosimo glejte tudi priporočila za odmerjanje zgoraj). Odmerki do 120 mg na dan so učinkoviti in na podlagi ocene kliničnih preskušanj tudi varni. Pri bolnikih z nezadostnim odgovorom na 60 mg odmerek lahko razmislimo o zvišanju odmerka do 90 mg ali 120 mg. Zvišanje odmerka mora biti osnovano na kliničnem odgovoru in prenosljivosti. Po ustaljenem odgovoru je za preprečitev relapsa priporočeno nadaljevanje zdravljenja še nekaj mesecev. *Bolečina diabetične periferne nevropatije:* Začetni in priporočeni vzdrževalni odmerek je 60 mg enkrat na dan s hrano ali brez nje. V kliničnih preskušanjih so z vidika varnosti vrednotili odmerjanja prek 60 mg enkrat na dan, do najvišjega odmerka 120 mg na dan, dajanega razdeljeno na enake odmerke. Plazemska koncentracija duloksetina izkazuje veliko različnost med posamezniki. Zato lahko nekaterim bolnikom, ki se na 60 mg odmerek ne odzovejo zadostno, koristi višji odmerek. Ödziv na zdravljenje je treba ovrednotiti po 2 mesecih. Pri bolnikih, ki imajo nezadosten uvodni odziv, dodatni odziv po preteku tega obdobja ni verjeten. Terapevtsko korist je treba redno ponovno ocenjevati (vsaj enkrat na vsake tri mesece). *Posebne populacije: Starejši bolniki:* Samo na podlagi starosti pri starejših bolnikih prilagajanje odmerka ni potrebno. Vendar pa je, kot pri vseh zdravilih, pri zdravljenju starejših bolnikov potrebna previdnost, posebno z odmerkom 120 mg duloksetina na dan za zdravljenje velike depresivne motnje ali generaliziranе anksiozne motnje, za kar je na voljo malo podatkov. *Okvarjeno delovanje jeter:* duloksetina pri bolnikih z obolenjem jeter, ki ima za posledico okvarjeno delovanje jeter, ne smemo uporabljati. *Okvarjeno delovanje ledvic:* Pri bolnikih z blago do zmerno okvarjenim delovanjem ledvic (očistek kreatinina 30 do 80 ml/min) odmerka ni treba prilagajati. Duloksetina ne smemo uporabljati pri bolnikih s hudo okvarjenim delovanjem ledvic (očistek kreatinina < 30 ml/min). *Pediatrična populacija:* Duloksetin se ne sme uporabljati za zdravljenje velike depresivne motnje pri otrocih in mladostnikih, mlajših od 18 let, zaradi pomislekov glede varnosti in učinkovitosti. Varnost in učinkovitost duloksetina za zdravljenje generaliziranе anksiozne motnje pri pediatričnih bolnikih, starih od 7 do 17 let, nista bili ugotovljeni. Varnost in učinkovitost duloksetina za zdravljenje bolečine periferne diabetične nevropatije nista bili dokazani. Podatkov ni na voljo. *Prekinitev zdravljenja:* Izogibati se moramo nenadni prekinitvi zdravljenja. Ob prenehanju zdravljenja z duloksetinom je treba odmerek zdravila v obdobju najmanj enega do dveh tednov postopoma zmanjševati, da zmanjšamo tveganje za pojav odtegnitvenih reakcij. V primeru, da se ob zmanjšanju odmerka ali prekinitvi zdravljenja pojavijo nevzdržni simptomi, je treba razmisliti o ponovni uvedbi prej predpisanega odmerka. Kasneje lahko zdravnik nadaljuje z zmanjševanjem odmerka, vendar bolj postopno. Način uporabe: Za peroralno uporabo. **KONTRAINDIKACIJE:** Preobčutljivost na zdravilno učinkovino ali katero koli pomožno snov. Duloksetina se ne sme uporabljati sočasno z neselektivnimi ireverzibilnimi zaviralci monoaminooksidaze (MAOI). Obolenje jeter, ki ima za posledico okvarjeno delovanje jeter. Duloksetin se ne sme uporabljati v kombinaciji s fluvoksaminom, ciprofloksacinom ali enoksacinom (tj. močnimi zaviralci CYP1A2), saj kombinacija povzroči povišane plazemske koncentracije duloksetina. Hudo okvarjeno delovanje ledvic (očistek kreatinina < 30 ml/min). Uvedba zdravljenja z duloksetinom je kontraindicirana pri bolnikih z nenadzorovano hipertenzijo, ker lahko izpostavi bolnike možnemu tveganju za hipertenzivno krizo. **POSEBNA OPOZORILA IN PREVIDNOSTNI UKREPI:** *Manija in epileptični napadi:* Duloksetin je treba pri bolnikih uporabljati previdno. *Midriaza:* pri predpisovanju duloksetina bolnikom s povišanim intraokularnim tlakom ali s tveganjem za akutni glavkom z zaprtim zakotjem je potrebna previdnost. *Krvni tlak in srčna frekvenca:* Duloksetin je bil pri nekaterih bolnikih povezan s povišanjem krvnega tlaka in klinično pomembno hipertenzijo. To je lahko posledica noradrenergičnega učinka duloksetina. Pri bolnikih z znano hipertenzijo in/ali drugimi srčnimi obolenji se priporoča spremljanje krvnega tlaka, zlasti v prvem mesecu zdravljenja. Pri bolnikih, ki so doživeli vztrajno povišanje krvnega tlaka medtem, ko so prejemali duloksetin, je potrebno razmisliti o zničanju odmerka ali postopni ukinitvi. Duloksetina ne smemo uvesti pri bolnikih z nenadzorovano hipertenzijo. *Okvarjeno delovanje ledvic:* Pri bolnikih s hudo okvarjenim delovanjem ledvic na hemodializi se pojavijo povišane plazemske koncentracije duloksetina. *Serotoninski sindrom:* lahko se pojavi potencialno življenjsko ogrožujoče stanje, še posebej pri sočasni uporabi drugih serotoninergičnih zdravil (vključno s SSRI, SNRI, tricikličnimi antidepresivi ali triptani), zdravil, ki vplivajo na presnovo serotonina, zaviralcev MAO, antipsihotikov ali drugih dopaminskih antagonistov, ki lahko vplivajo na serotoninergične nevrotransmiterske sisteme. Simptomi serotoninskega sindroma vključujejo spremembe duševnega stanja (npr. agitacijo, halucinacije, komo), avtonomno nestabilnost (npr. tahikardijo, spremenljiv krvni tlak, povišano telesno temperaturo), živčnomišične motnje (npr. hiperrefleksijo, motnje koordinacije) in/ali prebavne simptome (npr. navzejo, bruhanje, drisko). Pri sočasnem zdravljenju z duloksetinom in drugimi serotoninergičnimi zdravili, je priporočljivo bolnika skrbno nadzirati, še posebej med uvajanjem zdravljenja in pri zvišavanju odmerkov. Šentjanževka: Ob sočasni uporabi pripravkov rastlinskega izvora, ki vsebujejo šentjanževko (*Hypericum perforatum*), so lahko neželeni učinki pogostejši. *Samomor: Velike depresivne motnje in generaliziranа anksiozna motnja:* depresija je povezana z večjim tveganjem za pojav samomorilnih misli, samopoškodovanje in samomorilnosti. Takšno tveganje ostaja vse dokler ne pride do znatnega izboljšanja zdravstvenega stanja. V zgodnji fazi izboljšanja, se tveganje za samomor lahko poveča. *Druga psihiatrična stanja:* lahko so povezana z večjim tveganjem za pojav dogodkov povezanih s samomorom. Ta stanja so lahko sočasna z veliko depresivno motnjo. Bolnike s samomorom povezanimi dogodki v anamnezi, ali bolnikih, ki kažejo znatno stopnjo samomorilne miselnosti je treba pred uvedbo zdravljenja, med zdravljenjem in pri vsaki spremembi odmerka skrbno spremljati in nadzirati. Bolnike (in njihove skrbnike) je treba opozoriti, da morajo biti pozorni na kakršnokoli klinično poslabšanje, pojav samomorilnega vedenja, misli na samomor in pojav neobičajnih vedenjskih sprememb, ter da se morajo v primeru, da takšni simptomi se minejo, nemudoma posvetovati z zdravnikom. *Bolečina diabetične periferne nevropatije:* med zdravljenjem z duloksetinom ali kmalu po prenehanju zdravljenja so poročali o posameznih primerih samomorilnih misli in samomorilnega vedenja. *Krvavitev:* pri zdravljenju z duloksetinom so poročali o neormalnih krvavitvah, kot so ekhimoze, purpure in krvavitve iz prebavil. Pri bolnikih, ki jemljejo zdravila proti strjevanju krvi in/ali druga zdravila, ki vplivajo na delovanje trombocitov (npr. nesteroidne protivnetne učinkovine (NSAID) ali acetilsalicilna kislina), ter pri bolnikih z znano nagnjenostjo h krvavitvam, je potrebna previdnost. *Hiponatriemija:* je lahko posledica sindroma neustreznega izločanja antidiuretičnega hormona. Previdnost je potrebna pri starejših bolnikih, bolnikih s cirozo, dehidriranih bolnikih ali bolnikih, ki jemljejo diuretike. *Prekinitev zdravljenja:* ob nenadni prekinitvi zdravljenja se lahko pojavijo odtegnitveni simptomi. Svetujemo, da se ob prekinjanju zdravljenja odmerek duloksetina postopoma zmanjšuje v obdobju ne manj kot 2 tednov. *Starejši bolniki:* Pri zdravljenju starejših bolnikov je potrebna previdnost pri maksimalnem odmerjanju. *Akatizija/psihomotorični nemir:* Za bolnike s simptomi akatizije/psihomotoričnega nemira je večanje odmerka lahko škodljivo. *Zdravila, ki vsebujejo duloksetin:* Izogibati se je treba sočasni uporabi več kot enega od zdravil, ki vsebujejo duloksetin. *Hepatitis/povišane vrednosti jetrnih encimov:* Pri bolnikih, ki jemljejo zdravila, ki lahko povzročijo okvaro jeter, je pri uporabi duloksetina potrebna previdnost. Zdravilo *Duloksetin Sandoz 30 mg in 60mg vsebuje laktozo:* Bolniki z redko dedno intoleranco za fruktozo, malabsorpcijo glukoze/galaktoze ali s pomanjkanjem saharoza- izomaltaze ne smejo jemati tega zdravila. Zdravilo Duloksetin Sandoz 30 mg in 60 mg vsebuje barvilo alurno rdeče (E 129), *ki lahko povzroči alergijske reakcije. Zdravilo Duloksetin Sandoz 60 mg vsebuje barvilo sončno rumeno FCF (E 110),* ki lahko povzroči alergijske reakcije. *Uporaba pri otrocih in mladostnikih, mlajših od 18 let:* duloksetina pri zdravljenju otrok in mladostnikov, mlajših od 18 let, ne smemo uporabljati. **MEDSEBOJNO DELOVANJE Z DRUGIMI ZDRAVILI IN DRUGE OBLIKE INTERAKCIJ:** *Zaviralci monoaminooksidaze (MAOI):* zaradi nevarnosti za serotoninski sindrom duloksetina ne smemo uporabljati v kombinaciji z neselektivnimi ireverzibilnimi zaviralci monoaminooksidaze (MAOI), v 14 dneh po prenehanju zdravljenja z MAOI. Po prenehanju jemanja duloksetina mora preteči vsaj 5 dni pred začetkom jemanja MAOI. Sočasno uporabo duloksetina z moklobemidom odsvetujemo. Antibiotika linezolid bolniki, zdravljeni z duloksetinom, ne smejo prejemati. *Zaviralci CYP1A2:* sočasna uporaba duloksetina z močnimi zaviralci CYP1A2 (npr. fluvoksamin) povzroči višje koncentracije duloksetina. *Zdravila, ki delujejo na osrednje živčevje:* pri jemanju duloksetina v kombinaciji z drugimi centralno delujočimi zdravili ali snovmi, vključno z alkoholom in pomirjevali (npr. benzodiazepini, morfinomimetiki, antipsihotiki, fenobarbitalom, sedativnimi antihistaminiki), je potrebna previdnost. *Serotoninergična zdravila:* pri uporabi duloksetina sočasno s serotoninergičnimi zdravili (SSRI, SNRI, s tricikličnimi antidepresivi, kot je klomipramin ali amitriptilin, z MAOI, kot je moklobemid ali linezolid, s šentjanževko (*Hypericum perforatum*), triptani, tramadolom, petidinom in triptofanom) priporočamo previdnost. *Zdravila, ki jih presnavlja CYP2D6:* če se duloksetin daje sočasno z zdravili, ki jih presnavlja CYP2D6 (risperidon, triciklični antidepresivi in zdravila, ki imajo ozek terapevtski indeks (kot flekainid, propafenon in metoprolol), svetujemo previdnost. *Antikoagulanti in antitrombotična zdravila:* zaradi možnega povečanega tveganja za krvavitev, je potrebna previdnost pri kombinaciji duloksetina s peroralnimi antikoagulanti ali antitrombotičnimi zdravili. **NEŽELENI UČINKI:** *Zelo pogosti so;* glavobol, zaspanost, navzea, suha usta. *Pogosti so;* zmanjšanje apetita, nespečnost, agitacija, zmanjšanje libida, anksioznost, nenormalen orgazem, nenavadne sanje, omotica, letargija, tremor, parestezija, zamegljen vid, tinitus, palpitacije, zvišan krvni tlak, rdečica, zehanje, zaprtje, driska, bolečina v trebuhu, bruhanje, dispepsija, napenjanje, povečano znojenje, izpuščaj, mišično-skeletna bolečina, mišični krči, disurija, polakiurija, erektilna disfunkcija, motnje ejakulacije, zapoznela ejakulacija, padci, utrujenost, zmanjšanje telesne mase. Drugi manj pogosti neželeni učinki so navedeni v SmPC. **NAČIN IN REŽIM IZDAJE ZDRAVILA:** Rp/Predpisovanje in izdaja zdravila je le na recept. **OPREMA:** 28 trdih gastrorezistentnih kapsul v pretisnem omotu, v škatli. **IMETNIK DOVOLJENJA ZA PROMET:** Sandoz d.d., Ljubljana, Verovškova 57, 1000 Ljubljana, Slovenija. **INFORMACIJA PRIPRAVLJENA:** januar 2017 (ref.: 18.12.2015). **Pred predpisovanjem ali izdajanjem zdravila Duloksetin Sandoz, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila, ki je na voljo na www.lek.si/vademekum.**

**Vira: 1.** Cipriani A et al. Duloxetine versus other anti-depressive agents for depression (Review). 2012 The Cochrane Collaboration. Published by JohnWiley & Sons, Ltd.: 1-193. **2.** Gaynor PJ. Duloxetine versus placebo in the treatment of major depressive disorder and associated painful physical symptoms: a replication study. Current Medical Research and Opinion; Vol. 27, No. 10, 2011: 1859-1867.

**SAMO ZA STROKOVNO JAVNOST**
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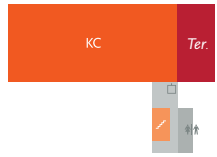
## SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA:

**Ime zdravila:** AZILECT 1 mg tablete. **Kakovostna in količinska sestava:** Ena tableta vsebuje 1 mg razagilina (kot mesilat). **Povzetek terapevtskih indikacij:** Zdravilo AZILECT je indicirano za zdravljenje idiopatske Parkinsonove bolezni (PB) kot monoterapija (brez levodope) ali kot dodatna terapija (z levodopo) pri bolnikih z nihanji končnih odmerkov. **Odmerjanje in način uporabe:** Razagilin se daje peroralno v odmerku 1 mg enkrat dnevno, z ali brez levodope. Lahko se jemlje s hrano ali brez nje. *Starejši:* za starejše bolnike ni potrebno spreminjanje odmerka. *Pediatrična populacija:* Uporaba zdravila AZILECT pri otrocih in mladostnikih ni priporočljiva zaradi nezadostnih podatkov o varnosti in učinkovitosti. *Bolniki z jetrno okvaro:* Uporaba razagilina pri bolnikih s hudo jetrno okvaro je kontraindicirana. Uporabi razagilina pri bolnikih z zmerno jetrno okvaro se je potrebno izogibati. Ob začetku zdravljenja z razagilinom je pri bolnikih z blago jetrno okvaro potrebna previdnost. V primeru, da pri bolniku blaga jetrna okvara napreduje do zmerne, se mora zdravljenje z razagilinom prekiniti. *Bolniki z ledvično okvaro:* Pri ledvičnih okvarah ni potrebno spreminjati odmerka. **Povzetek kontraindikacij**: Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Sočasno zdravljenje z drugimi inhibitorji monoaminooksidaze (MAO) (vključno z zdravili in naravnimi pripravki brez recepta kot je npr. šentjanževka) ali s petidinom. Najmanj 14 dni mora preteči med prekinitvijo zdravljenja z razagilinom in začetkom zdravljenja z inhibitorji MAO ali s petidinom. Razagilin je kontraindiciran pri bolnikih s hudo jetrno okvaro. **Povzetek posebnih opozoril in previdnostnih ukrepov**: Sočasni uporabi razagilina in fluoksetina ali fluvoksamina se je potrebno izogibati. Od prekinitve zdravljenja s fluoksetinom in do začetka zdravljenja z razagilinom mora preteči najmanj pet tednov. Najmanj 14 dni pa mora preteči med prekinitvijo zdravljenja z razagilinom in do začetka zdravljenja s fluoksetinom ali fluvoksaminom. Pri bolnikih, ki se zdravijo z agonisti dopamina in/ali dopaminergičnimi zdravljenji, lahko pride do motenj pri obvladovanju impulzov. Prav tako so bila podobna poročila glede motenj pri obvladovanju impulzov prejeta v obdobju trženja razagilina. Bolnike je treba redno spremljati zaradi možnosti razvoja motenj pri obvladovanju impulzov. Bolnike in njihove skrbnike je treba opozoriti na vedenjske simptome motenj pri obvladovanju impulzov, ki so bili opaženi pri bolnikih, ki so se zdravili z razagilinom, kar vključuje primere kompulzije, obsesivne misli, patološkega hazardiranja, povečanega libida, hiperseksualnosti, impulzivnega vedenja in kompulzivnega zapravljanja ali nakupovanja. Ker razagilin poveča učinke levodope, se lahko neželeni učinki levodope povečajo in obstoječa diskinezija poslabša. Ta neželeni učinek se lahko izboljša z zmanjšanjem odmerka levodope. Poročali so o hipotenzivnih učinkih pri sočasni uporabi rasagilina z levodopo. Bolniki s Parkinsonovo boleznijo so še posebej ranljivi na neželene učinke hipotenzije zaradi obstoječe problematike s hojo. Sočasna uporaba razagilina in dekstrometorfana ali simpatikomimetikov, kot so tisti, ki so prisotni v nazalnih in peroralnih dekongestivih ali zdravil proti prehladu, ki vsebujejo efedrin ali psevdoefedrin, ni priporočljiva. V času programa kliničnega razvoja je pojavnost primerov melanoma nakazovala možnost povezave z razagilinom. Zbrani podatki nakazujejo, da je Parkinsonova bolezen, ne pa zdravila sama po sebi, povezana z visokim tveganjem karcinoma kože (ne izključno melanoma). Vsake sumljive kožne lezije mora oceniti specialist. Pri bolnikih z blago jetrno okvaro je ob začetku zdravljenja z razagilinom potrebna previdnost. Uporabi razagilina pri pacientih z zmerno jetrno okvaro se je potrebno izogibati. V primeru, da pri bolniku blaga jetrna okvara napreduje do zmerne, se mora zdravljenje z razagilinom prekiniti. **Povzetek medsebojnega delovanja z drugimi zdravili in druge oblike interakcij**: Obstajajo številne znane interakcije med neselektivnimi inhibitorji MAO in drugimi zdravili. Razagilin se ne sme uporabljati sočasno z drugimi inhibitorji MAO (vključno z zdravili in naravnimi pripravki brez recepta kot je npr. šentjanževka), ker obstaja tveganje, da neselektivna inhibicija monoaminooksidaze (MAO) lahko vodi do hipertenzivnih kriz. Ob sočasni uporabi petidina in inhibitorjev MAO, vključno z drugimi selektivnimi inhibitorji MAO-B, so poročali o resnih neželenih učinkih. Sočasna uporaba razagilina in petidina je kontraindicirana. Obstajajo poročila o medsebojnih interakcijah zdravil pri sočasni uporabi inhibitorjev MAO in simpatikomimetičnimi zdravili. Zaradi inhibitorne monoaminooksidazne aktivnosti razagilina sočasna uporaba razagilina in simpatikomimetikov, kot so tisti, ki so prisotni v nazalnih in peroralnih dekongestivih ali zdravil proti prehladu, ki vključujejo efedrin ali psevdoefedrin, ni priporočljiva. Obstajajo poročila o interakcijah zdravil pri sočasni uporabi dekstrometorfana in neselektivnih inhibitorjev MAO. Zaradi inhibitorne monoaminooksidazne aktivnosti razagilina sočasna uporaba razagilina in dekstrometorfana ni priporočljiva. Sočasni uporabi razagilina in fluoksetina ali fluvoksamina se je potrebno izogibati. Za sočasno uporabo razagilina in selektivnih inhibitorjev ponovnega privzema serotonina (SSRI)/selektivnih inhibitorjev ponovnega privzema serotonina-norepinefrina (SNRI) v kliničnih študijah. Poročali so o resnih neželenih učinkih ob sočasni uporabi SSRI, SNRI, tricikličnih in tetracikličnih antidepresivov in inhibitorjev MAO. Zaradi tega je s stališča inhibitorne monoaminooksidazne aktivnosti razagilina potrebno antidepresive uporabljati previdno. Pri bolnikih s Parkinsonovo boleznijo, ki so kot dodatno terapijo stalno prejemali levodopo, ni bilo nobenega klinično pomembnega vpliva levodope na očistek razagilina. *In vitro* študije presnove so pokazale, da je citokrom P450 1A2 (CYP1A2) glavni encim, ki je odgovoren za presnavljanje razagilina. Sočasna uporaba razagilina in ciprofloksacina (inhibitor CYP1A2) je povečala AUC razagilina za 83 %. Sočasna uporaba razagilina in teofilina (substrat CYP1A2) ni vplivala na njuno farmakokinetiko. Močni inhibitorji CYP1A2 lahko torej spremenijo plazemske ravni razagilina, zaradi česar se morajo uporabljati previdno. Obstaja možnost, da so pri kadilcih znižane plazemske ravni razagilina zaradi indukcije metabolnega encima CYP1A2. *In vitro* študije so pokazale, da razagilin v koncentraciji 1 µg/ml (ekvivalent ravni, ki je 160-kratna povprečna $C_{max}$ ~ 5,9 – 8,5 ng/ml pri bolnikih s Parkinsonovo boleznijo po zaužitju večkratnih odmerkov 1 mg razagilina) ne inhibira izoencimov citokroma P450: CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 in CYP4A9. Glede na te rezultate ni verjetno, da bi terapevtske koncentracije razagilina klinično pomembno součinkovale s substrati teh encimov. Sočasna uporaba razagilina in entakapona poveča oralni očistek razagilina za 28 %. *Interakcije tiramin/razagilin*: Rezultati petih izmenjalnih študij s tiraminom (pri prostovoljcih in bolnikih s Parkinsonovo boleznijo), skupaj z rezultati spremljanja krvnega pritiska po obrokih hrane v domačem okolju (464 bolnikov zdravljenih z 0,5 ali 1 mg/dan razagilina ali placeba kot dodatne šest mesečne terapije k levodopi brez omejitve tiramina) in dejstva, da ni poročil o interakcijah tiramin/razagilin v kliničnih študijah, ki so bile izvajane brez omejitve tiramina, kažejo na to, da se lahko razagilin varno uporablja brez dietne omejitve tiramina. **Plodnost, nosečnost in dojenje**: Za razagilin ni na voljo kliničnih podatkov od nosečnic, ki so bile izpostavljene zdravilu. Študije na živalih ne kažejo na neposredne ali posredne škodljive vplive na nosečnost, razvoj zarodka/plodu, porod ali postnatalni razvoj. Pri predpisovanju zdravila nosečnicam je potrebna previdnost. Eksperimentalni podatki kažejo, da razagilin zavira izločanje prolaktina, to pa lahko zavre dojenje. Ni znano, ali se razagilin izloča v materino mleko. Ob uporabi zdravila pri doječih materah je potrebna previdnost. **Vpliv na sposobnost vožnje in upravljanja s stroji:** Študij o vplivu na sposobnost vožnje in upravljanja s stroji niso izvedli. Bolnike je potrebno opozoriti, naj ne upravljajo nevarnih strojev, vključno z motornimi vozili, dokler niso ustrezno prepričani, da zdravilo AZILECT na njih ne vpliva škodljivo. **Povzetek neželenih učinkov:** Neželeni učinki z najmanj 2 % razliko v primerjavi s placebom pri monoterapiji: Gripa, depresija, halucinacije, glavobol, konjunktivitis, rinitis, dermatitis, mišično skeletna bolečina v vratu, bolečina v vratu. Neželeni učinki z najmanj 2 % razliko v primerjavi s placebom pri dodatni terapiji: Diskinezija, ortostatična hipotenzija, bolečina v trebuhu, zaprtje, siljenje na bruhanje, bruhanje, zmanjšanje telesne mase. V obdobju trženja so pri bolnikih, ki so jemali razagilin, poročali o primerih zvišanega krvnega tlaka, vključno z redkimi primeri o hipertenzivni krizi, povezani z zaužitjem neznane količine s tiraminom bogatih jedi. V obdobju trženja je prišlo do enega primera zvišanega krvnega tlaka pri bolniku, ki je med jemanjem razagilina uporabljal očesni vazokonstriktor tetrahidrozolin. **Preveliko odmerjanje:** Ne obstaja specifičen antidot. V primerih prevelikega odmerjanja je potrebno bolnika opazovati ter uvesti ustrezno simptomatsko in podporno zdravljenje. **Način in režim predpisovanja in izdaje zdravila:** Predpisovanje in izdaja zdravila je le na recept. **Imetnik dovoljenja za promet:** Teva B.V., Swensweg 5, 2031 GA Haarlem, Nizozemska. **Datum zadnje revizije besedila:** 22. februar 2016.

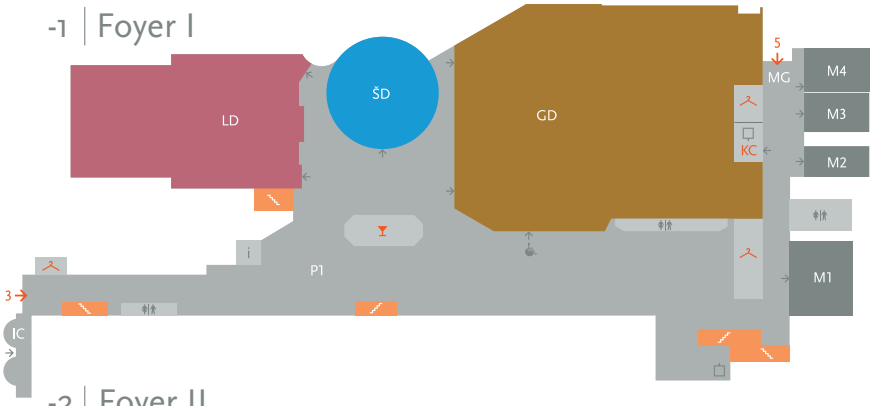
Datum priprave informacije: maj 2017. Samo za strokovno javnost. Način in režim predpisovanja in izdaje zdravila: Rp.
Za podrobnejše informacije o zdravilu, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila, ki je objavljen na spletni strani Evropske agencije za zdravila (www.ema.europa.eu) ali se obrnite na zastopnika imetnika dovoljenja za promet z zdravilom v Sloveniji, Pliva Ljubljana d.o.o., Pot k sejmišču 35, 1231 Ljubljana-Črnuče, tel.: 01 58 90 399, e-mail: info@tevasi.si.
SN/AZT/17/0002

## 6 | CD Club

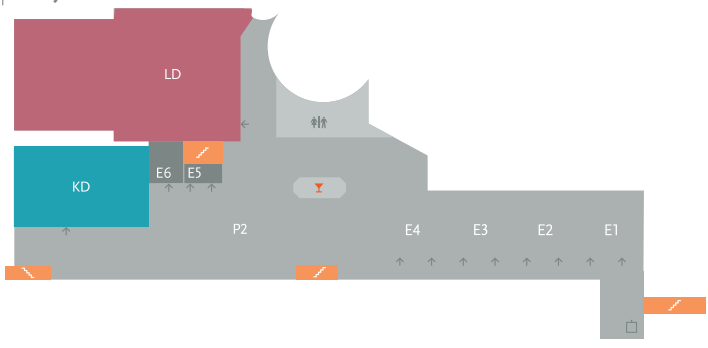



## 0 | Grand Reception Hall


## -1 | Foyer I




## -2 | Foyer II




### 6 | CD Club

- KC — CD Club *from Erjavčeva St., Entrance 5*
- *Ter.* — Terrace

### 0 | Ground Floor

- 1 — Entrance 1 *Prešernova St.*
- 2 — Entrance 2 *Republic Sq.*
- 4 — Entrance 4 *Stage Ent.*
- 6 — Entrance 6 *Employee Ent.*
- VS — Grand Reception Hall
- GD — Gallus Hall *Circle and Balcony*
- GC — CD Galery
- LN — Lili Novy Club *Mezzanine*
- DP — Duša Počkaj Hall
- *Rec.* — Reception
- Bar »Idealist«

### -1 | Foyer I

- 3 — Entrance 3 *from Maxi Pasgeway*
- 5 — Entrance 5 *from Erjavčeva St.*
- P1 — Foyer I
- GD — Gallus Hall *Stalls*
- LD — Linhart Hall *Balcony*
- ŠD — Štih Hall
- MG — Small Gallery
- IC — Info Centre & Box Office
- KC — CD Club *on 6th Floor*
- M — Conference Rooms 1–4

### -2 | Foyer II

- LD — Linhart Hall *Stalls*
- KD — Kosovel Hall
- P2 — Foyer II
- E — Conference Rooms 1–6

- Toilette
- Cloakroom
- Lift
- Bar
- CD Entrance
- Hall Entrance
- Stairway