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ISTITUTO AUXOLOGICO ITALIANO
Istituto di ricovero e cura a carattere scientifico

ENCALS
European Network for the Cure of ALS
Meeting 2016

Programme and Abstracts

May 19th - May 21st 2016
The University of Milan, Italy

UNIVERSITÀ DEGLI STUDI
DI MILANO

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MESSAGE FROM THE ORGANIZERS

Dear Participants,

We welcome you to the University of Milan for the 14th meeting of ENCALS.

This meeting is a forum for the European ALS community, but the main goal is to encourage young researchers to present their data, and to meet and interact with more established members of the ALS community.

ALS remains a terrible disease and we are desperately aiming for a cure. We are pleased to open the ENCALS Meeting in Milan with Prof. Luigi Naldini and his message of hope after completing a successful gene therapy in humans.

We are delighted to have as our guests eminent researchers from both sides of the Atlantic, including Prof. John E. Landers, Prof. Merit E. Cudkowicz, Prof. Don W. Cleveland, and Prof. Christine Vande Velde.

The oral sessions this year will focus on Genes and Genomics, Clinical aspects, Therapeutic approaches, and Disease mechanisms and modeling.

As usual for ENCALS, the quality of the submitted abstracts is excellent, and both platform and poster sessions will provide ample opportunity to share new ideas and discuss the exciting developments in the field.

We gratefully acknowledge the support from Biogen, Cytokinetics, Orion Pharma and the administrative support from the University of Milan and IRCCS Istituto Auxologico Italiano.

We thank the members of the scientific committee (Orla Hardiman, Severine Boillée, Caterina Bendotti, Jochen Weishaupt, Federica Agosta), Akke Albada (Communications officer at the University Medical Centre Utrecht) for her excellent administrative support of ENCALS and Patrizia Nelli, Antonia Ratti, Nicola Ticozzi, and Luca Grappiolo, who have undertaken most of the local organization with BioMedia.

We look forward to an exciting meeting, and hope that you will also find time to explore some of the treasures of our historic University and enjoy the vibrant life of Milan.

Professor Vincenzo Silani
On behalf of the organizing committee
University of Milan Medical School
IRCCS Istituto Auxologico Italiano
Milan

The ENCALS Executive Committee

Chair

Leonard van den Berg (The Netherlands)

Vice Chair

Orla Hardiman (Ireland)

Members

Peter Andersen (Sweden)

Adriano Chiò (Italy)

Julian Grosskreutz (Germany)

Magdalena Kuzma (Poland)

Albert Ludolph (Germany)

Jesus Mora Pardina (Spain)

Wim Robberecht (Belgium)

Francois Salachas (France)

Pamela Shaw (England)

Kevin Talbot (England)

Markus Weber (Switzerland)

DAY 1 - Thursday 19th May 2016

12:00-13:30	Registration and lunch
13:30-13:45	Opening session *Prof. Gianluca Vago, Rector of the University of Milan*
13:45-14:15	Opening lecture *Prof. Luigi Naldini*
14:15-16:00	Session 1a: Genes and Genomics
16:00-16:30	Coffee break
16:30-17:30	Session 1b: Genes and Genomics
17:30-19:00	Poster Session - *wine and cheese aperitif*
19:00-20:00	Project MinE Meeting (closed meeting)
20:00	Cytokinetics Investigator Reception (closed meeting)

DAY 2 - Friday 20th May 2016

09:00-10:45	Session 2a: Clinical aspects
10:45-11:15	Coffee break
11:15-12:30	Session 2b: Clinical aspects
12:30-14:15	Lunch and Poster session
13:00-14:15	ENCALS Board meeting (closed meeting)
14:15-16:00	Session 3a: Therapeutic approaches
16:00-16:30	Coffee break
16:30-17:30	Session 3b: Therapeutic approaches
17:30-19:00	Poster Session - *wine aperitif*
20:00	ENCALS Dinner

DAY3 - SATURDAY 21st May 2016

08:30-08:45	Poster award for PhD students
08:45-09:00	ENCALS Young Investigator Award
09:00-11:00	Session 4a: ALS disease mechanisms and modeling
11:00-11.30	Coffee break
11:30-13:00	Session 4a: ALS disease mechanisms and modeling
13:00-13:15	Closing of the Meeting
13:15-19:00	TRICALS Workshop Outcome Measures

DAY4 - SUNDAY 22nd May 2016

09:00-13:00	TRICALS Workshop Outcome Measures

DAY 1 - Thursday, 19th May 2016

Venue: Main Lecture Hall - University of Milan Central Campus
University of Milan Medical School, Via Festa del Perdono 7, Milan

12:00-13:30 Registration and Lunch

13:30-13:45 **Opening Session**
Chairs: Prof. Vincenzo Silani & Prof. Leonard Van den Berg
Prof. Gianluca Vago, Rector of the University of Milan

13:45-14:15 **Opening Lecture**
Invited Speaker: Prof. Luigi Naldini (Italy)
Director SR-TIGET, San Raffaele Telethon Institute for Gene Therapy
"Gene Therapy with Hematopoietic Stem Cells: from bench to bedside"

ORAL SESSION 1a: GENES and GENOMICS
Chairs: Prof. Albert Ludolph, Prof. Jan Veldink

14:15-15:00 **State of the Art**
Invited speaker - Prof. John E. Landers (USA)
"Transitioning Discoveries in ALS Genetics to Therapeutic Treatments"

15:00-15:15 **OS.1:** Project MinE GWAS: Novel risk variants and the genetic architecture of ALS
Wouter van Rheenen (The Netherlands)

15:15-15:30 **OS.2:** What do we really know about TBK1 mutations in neurodegenerative diseases?
Axel Freischmidt (Germany)

15:30-15:45 **OS.3:** An optineurin insufficiency model of amyotrophic lateral sclerosis
Ivana Munitic (Croatia)

15:45-16:00 **OS.4:** Identification of novel genetic risk factors in sporadic ALS, a discordant monozygotic twin approach
Gijs Tazelaar (The Netherlands)

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

ORAL SESSION 1b: GENES and GENOMICS
Chairs: Prof. John E. Landers, Dr. Nicola Ticozzi

16:30-16:45 **OS.5:** Latent Cluster Analysis of ALS Phenotypes and GWAS: Identification of Prognostically Differing Groups
William Sproviero (UK)

16:45-17:00 **OS.6:** Hidden Treasures: hunting for cryptic splicing resulting from the knockdown of TDP-43
Jack Humphrey (UK)

17:00-17:15 **OS.7:** Genetic modifier screens link nuclear transport defects to DPR pathology in C9orf72 ALS/FTD
Steven Boeynaems (Belgium)

17:15-17:30 **OS.8:** Comparative interactomics analysis of several ALS-associated proteins identifies converging molecular pathways
R. Jeroen Pasterkamp (The Netherlands)

17:30-19:00 **POSTER SESSION I (Odd Numbers) - *wine and cheese aperitif***

19:00-20:00 **Project MinE (closed meeting)**

20.00 **Cytokinetics Investigator Reception (closed meeting)**

DAY 2 - Friday, 20th May 2016

Venue: Main Lecture Hall - University of Milan Central Campus
University of Milan Medical School, Via Festa del Perdono 7, Milan

ORAL SESSION 2a: CLINICAL ASPECTS
Chairs: Prof. Orla Hardiman, Prof. Adriano Chiò

09:00-09:45 **State of the Art**
Invited speaker: Prof. Dr. Merit E. Cudkowicz (USA)
"ALS Trials and Trialists: new opportunities and approaches to finding treatments for our patients"

09:45-10:00 **OS.9:** Development and external validation of a model for prediction of survival in individual ALS patients
Henk-Jan Westeneng (The Netherlands)

10:00-10:15 **OS.10:** Euro-MOTOR: a case-control study of fitness measures as risk factors for ALS
Anne Visser (The Netherlands)

10:15-10:30 **OS.11:** Quality control of Motor Unit Number Index in 6 muscles in a single-subject "Round-Robin" setup
Christoph Neuwirth (Switzerland)

10:30-10:45 **OS.12:** Assessing long-term G-CSF as treatment option in ALS
Siw Johannesen (Germany)

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

ORAL SESSION 2b: CLINICAL ASPECTS
Chairs: Prof. Markus Weber, Dr. Christian Lunetta

11:15-11:30 **OS.13:** Novel phenotypic subgroups associated with the C9orf72 expansion and survival in European ALS cohort
James Rooney (Ireland)

11:30-11:45 **OS.14:** Structural and functional brain signatures of C9orf72 in amyotrophic lateral sclerosis
Federica Agosta (Italy)

11:45-12:00 **OS.15:** Neuropathological spreading patterns in ALS analyzed by multiparametric MRI: DTI and 'resting-state'
Jan Kassubek (Germany)

12:00-12:15 **OS.16:** A connectivity-based analysis of striatal pathology in ALS
Peter Bede (Ireland)

12:15-12:30 **OS.17:** In vivo analysis of neuro-inflammation with [11C]-PBR28: Clinical and MR spectroscopic correlations
Matthew Evans (UK)

12:30-14:15 **LUNCH and POSTER SESSION**

13:00-14:15 **ENCALS Board meeting (closed meeting)**

ORAL SESSION 3a: THERAPEUTIC APPROACHES
Chairs: Prof. Ludo van den Bosch, Prof. Angelo Poletti

14:15-15:00 **State of the Art**
Invited speaker: Prof. Don W. Cleveland (USA)
"Gene silencing therapy for ALS and beyond"

15:00-15:15 **OS.18:** Structure and small molecule interaction of pathogenic C9orf72 RNA
Roberto Simone (UK)

15:15-15:30 **OS.19:** CRISPR/Cas9 genome editing results in correction of ALS phenotypes in C9orf72 mutant iPSC-derived motor neurons
Nidaa Ababneh (UK)

15:30-15:45 **OS.20:** Oculomotor restricted protein SYT13 protects motor neuron from selective death in ALS and SMA
Stefania Corti (Italy)

15:45-16:00 **OS.21:** Detailed analysis of misfolded SOD1 species in patient-derived cell types
Elin Forsgren (Sweden)

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

ORAL SESSION 3b: THERAPEUTIC APPROACHES
Chairs: Dr. Caterina Bendotti, Prof. Susanne Petri

16:30-16:45 **OS.22:** Targeting extracellular cyclophilin A extends survival in the SOD1G93A mouse model of ALS
Valentina Bonetto (Italy)

16:45-17:00 **OS.23:** CSF1R blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasion of macrophages into peripheral nerves
Anna Martinez-Muriana (Spain)

17:00-17:15 **OS.24:** Dimethyl fumarate (Tecfidera) delays onset and improves motor function in SOD1G93A transgenic mouse
Richard Mead (UK)

17:15-17:30 **OS.25:** Beneficial effects of RNS60 in cellular and animal models of amyotrophic lateral sclerosis
Antonio Vallarola (Italy)

17:30-19:00 **POSTER SESSION II (Even Numbers) - *wine apertif***

20:00 **ENCALS DINNER**
Casa Cardinale Ildefonso Schuster
University of Milan Central Campus
Via Sant'Antonio 5, Milan

DAY 3 - Saturday, 21st May 2016

Venue: Casa Cardinale Ildefonso Schuster – University of Milan Central Campus, Via Sant'Antonio 5, Milan

08:30-08:45 **POSTER AWARD FOR PhD STUDENTS**

08:45-09:00 **ENCALS YOUNG INVESTIGATOR AWARD**

ORAL SESSION 4a: ALS DISEASE MECHANISMS AND MODELING
Chairs: Dr. Severine Boillée, Dr. Georg Haase

09:00-09:45 **State of the Art**
Invited speaker: Prof. Christine Vande Velde (Canada)
"Cell biological mechanisms in ALS pathogenesis"

9:45-10:00 **OS.26:** Approaches to enhance the cell response to proteotoxicity in ALS
Valeria Crippa (Italy)

10:00-10:15 **OS.27:** Phenotypic profiling of compartmentalized iPSC-derived ALS neurons with live imaging tools
Arun Pal (Germany)

10:15-10:30 **OS.28:** Evaluation of a TDP-43Q331K mouse model: is it useful for therapeutic trials in ALS?
Jodie Stephenson (UK)

10:30-10:45 **OS.29:** Alpha-synuclein interacts with SOD1 and promotes its oligomerization
Anika Marie Helferich (Germany)

10:45-11:00 **OS.30:** Two superoxide dismutase prion strains transmitting amyotrophic lateral sclerosis
Elaheh Ekhtiari Bidhendi (Sweden)

11:00-11:30 **Coffee break**

ORAL SESSION 4b: ALS DISEASE MECHANISMS AND MODELING
Chairs: Dr. Antonia Ratti, Dr. Boris Rogelj

11:30-11:45 **OS.31:** C9ORF72 function synergizes the toxicity of Ataxin-2 with intermediate polyQ repeats
Edor Kabashi (France)

11:45-12:00 **OS.32:** Direct RNA toxicity in a transient zebrafish model of C9orf72 ALS/FTD is abrogated by Purα
Bart Swinnen (Belgium)

12:00-12:15 **OS.33:** Depletion of C9orf72 causes immune system dysfunction in mice
Emma Sudria Lopez (The Netherlands)

12:15-12:30 **OS.34:** Role of FUS in post synaptic neuromuscular junction differentiation
Gina Picchiarelli (France)

12:30-12:45 **OS.35:** Cellular characterisation of a novel FUS mouse model of ALS
Nicol Birsa (UK)

12:45-13:00 **OS.36:** Drosophila FUS mutant phenotypes are mediated by increased Xrp1 expression in neurons
Erik Storkebaum (Germany)

13:00-13:15 **CLOSING OF THE MEETING**
Prof. Vincenzo Silani & Prof. Leonard Van den Berg

13:15-19:00 **TRICALS Workshop Outcome Measures**

DAY 4 - Sunday 22nd May 2016

Venue: Casa Cardinale Ildefonso Schuster – University of Milan Central Campus, Via Sant'Antonio 5, Milan

9:00-13:00 **TRICALS Workshop Outcome Measures**

Poster Session I (Odd number Abstracts)

Thursday 19th May, h.17.30-19.00

Biomarkers I (P1-P11)

P.01. Whole CSF proteome analysis for the identification of specific biomarkers in ALS

Muckova P 1,2, Wendler S 1, Prell T 2, Stubendorff B 2, Ringer TM 2, Hammer N 2, Heiling B 2, Rumpel M 2, Schenk A 2, Gunkel A 2, Witte OW 2,3, Rhode H 1, Grosskreutz J 2. 1 Institute for Biochemistry, Jena University Hospital, Nonnenplan 2, 07743 Jena, Germany, 2 Hans Berger Department of Neurology, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany 3 Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany

P.03. Microvesicles and exosomes in ALS: biomarkers for disease propagation and therapeutic targets

Sproviero D.1, La Salvia S. 2, Colombo F. 3, Giannini M. 2, Diamanti L. 4, Bini P. 4, Pansarasa O. 1, Porretti L. 3, Cereda C. 1, 1 Laboratory of Experimental Neurobiology,"C. Mondino" National Neurological Institute, Pavia, Italy. 2 Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy. 3 Clinical Chemistry and Microbiology Laboratory, Flow Cytometry and Experimental Hepatology Service, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. 4 Neurology Department, IRCCS National Neurological Institute C. Mondino, University of Pavia, via Mondino 2, 27100, Pavia, Italy.

P.05. Metal concentrations in cerebrospinal fluid and blood plasma from patients with ALS

Per M Roos, MD PhD Specialist in neurology and clinical neurophysiology Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden Department of Clinical Physiology, St.Goran Hospital, Stockholm, Sweden

P.07. Gene expression biomarkers in ALS patients: a study in accesible samples

Atencia, G. 1, Tapial, S. 1, Calvo, A.C. 1,2, Juarez-Rufian, A. 1, Cordero-Vazquez, P. 1, Marti-n, M.A. 3, Moraleda, J.M. 4, Martinez-Pérez S. 4, Esteban-Pérez, J. 1, Osta, R. 2 and Garci¬a-Redondo, A. 1, 1 Neurology department ALS Unit. CIBERER U-723. Health research Institute, October 12th University Hospital Avda. Cordoba s/n 28041 Madrid Spain. 2 LAGENBIO-I3A, Aragonese Institute of Health Sciences (IACS), Faculty of Veterinary, University of Zaragoza, Miguel Servet, 177. 50013 Zaragoza. Spain. 3 Biochemistry department. CIBERER U-723. Health research Institute, October 12th University Hospital â Avda. Cordoba s/n 28041 Madrid Spain. 4 Hematopoietic transplant Unit and cell Therapy. Hematology department. Virgen de la Arrixaca University Hospital â Ctra. Madrid-Cartagena, s/n 30120 Murcia Spain.

P.09. Clinical validation of pNfH and NfL as prognostic and diagnostic biomarkers for ALS

De Schaepdryver M.(1)*, Poesen K.(1)*, Stubendorff B.(2)*, Muckova P.(2,3), Wendler S.(3), Prell T.(2), Ringer TM.(2), Rhode H.(3), Couwelier G.(4), D'Hondt A.(4), Lamaire N.(4), Tilkin P.(4), Van Reyen D.(4), Gourmaud S.(5), Hammer N.(2), Heiling B.(2), Rumpel M.(2), Schenk A.(2), Gunkel A.(2), Witte OW.(2,6), Paquet C.(5), Vandenberghe R.(4,7), Grosskreutz J.(2)#, Van Damme P.(4,8)# * Shared first authorship # Shared last authorship (1) Laboratory for Molecular Neurobiomarker Research, KU Leuven (University of Leuven) and Laboratory Medicine, University Hospitals Leuven, Herestraat 49, 3000 Belgium (2) Hans Berger Department of Neurology, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany (3) Institute for Biochemistry, Jena University Hospital, Nonnenplan 2, 07743 Jena, Germany (4) University Hospitals Leuven, Department of Neurology, Herestraat 49, 3000 Leuven, Belgium (5) CMRR Paris Nord AP-HP, Groupe Hospitalier Lariboisière Fernand-Widal Saint-Louis, INSERM, U942, Université Paris Diderot, Sorbonne Paris Cité, UMRS 942, Paris, France. (6) Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany (7) Laboratory for Cognitive Neurology, KU Leuven (University of Leuven), Herestraat 49, 3000 Leuven, Belgium (8) KU Leuven - University of Leuven, Department of Neurosciences, VIB - Vesalius Research, Belgium

P.11. Blood Lead, Bone Turnover, and Survival in Amyotrophic Lateral Sclerosis

Fang Fang (a), Tracy L. Peters (a, b), John D. Beard (b, c), David M. Umbach (d), Jean Keller (e), Daniela Mariosa (a), Kelli D. Allen (f), Weimin Ye (a), Dale P. Sandler (b), Silke Schmidt (g), Freya Kamel (b) (a) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; (b) Epidemiology Branch, National Institute of Environmental Health Sciences, NIH, DHHS, United States; (c) Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States; (d) Biostatistics and Computational Biology Branch, National Institute of Environmental Health Sciences, NIH, DHHS, United States; (e) Westat, Durham, North Carolina, United States; (f) Epidemiology Research and Information Center, Durham VA Medical Center, Durham, North Carolina, United States and Department of Medicine & Thurston Arthritis Research Center, University of North Carolina at Chapel Hill, North Carolina, United States; (g) Department of Medicine, Duke University Medical Center, Durham, North Carolina, United States

Clinical aspects I (P13-P55)

P.13. The utility of multimodal imaging in the diagnosis of ALS

Pilar M. Ferraro,1 Federica Agosta,1 Nilo Riva,2 Massimiliano Copetti,4 Yuri Falzone,2 Adriano Chiò,5 Gianni Sorar,6 Giancarlo Comi,2 Andrea Falini,3 Massimo Filippi.1,2 1Neuroimaging Research Unit, 2Department of Neurology, and 3Department of Neuroradiology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy; 4Biostatistics Unit, IRCCS-Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy; 5ALS Center, Rita Levi Montalcini Department of Neuroscience, University of Torino, Torino, Italy; 6Department of Neurosciences, University of Padova, Padova, Italy

P.15. The construction of the Swedish MND quality registry
Ingre C(1,2), Regodon Wallin A(3), Samuelsson K(1,2), Press R(1,2), Zachau A(1,2), Fang F(3) and Ronnevi L-O(1,2) 1 Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden 2 Department of Neurology, Karolinska University Hospital, Stockholm, Sweden 3 Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

P.17. Telesurveillance in ALS patients: a 3 year center experience
Author: Andrea Calvo[1], Alessio Mattei[2], Cinzia Ferrero[2], Marco Bardessono[2], Giuseppe Tabbia[2], Lucia Selvaggi[2], Maurizio Cangianiello[3], Laura Dominici[3], Jacopo Bellinati[3], Caterina Bucca[2], Stefania Cammarosano[1], Antonio Ilardi[1], Antonio Canosa[1], Cristina Moglia[1], Adriano Chiò[1] [1]ALS Centre, "Rita Levi Montalcini" Department of Neuroscience, University of Torino, Torino, Italy; [2]Respiratory Unit, AOU Citta della Salute e della Scienza di Torino, Torino, Italy; [3]MedicAir Italia, MedicAir, Pogliano Milanese (MI), Italy

P.19. Structural brain MRI abnormalities in Kennedy's disease
Pilar M. Ferraro,1 Federica Agosta,1 Giorgia Querin,4 Nilo Riva,2 Cinzia Bertolin,4 Elisa Da Re,4 Massimiliano Copetti,5 Giancarlo Comi,2 Andrea Falini,3 Gianni SorarÃ¹,4 Massimo Filippi.1,2 1Neuroimaging Research Unit, 2Department of Neurology, Institute of Experimental Neurology, and 3Department of Neuroradiology and CERMAC, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy; 4Department of Neurosciences, University of Padova, Padova, Italy; 5Biostatistics Unit, IRCCS-Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy.

P.21. Selective white matter vulnerability in ALS: implications for diagnostic classification
Schuster Christina, Hardiman Orla, Bede Peter Academic, Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin, Ireland

P.23. Premorbid body mass index and its behavior during follow up is a predictor of survival in ALS
Andres Julian Paipa Merchan [1] Nuria Virgili [2] Imma Jimenez [2] Anna Prats [2] Veronica Herrera [2] Raul Dominguez Rubio [1] Janina Turon [1] Monica Povedano Panades [1] [1] Servei de Neurologia, IDIBELL-Hospital de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain. [2] Servei de Nutricié, IDIBELL-Hospital de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain.

P.25. Predicting Survival of ALS Patients from Diffusion-Weighted Images Using Deep Learning
Hannelore K. van der Burgh, MSc 1, Ruben Schmidt, MSc 1, Henk-Jan Westeneng, MD 1, Marcel A. de Reus, PhD 2, Leonard H. van den Berg*, MD PhD 1, Martijn P. van den Heuvel*, PhD 2 * authors contributed equally Affiliations 1. Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, Netherlands 2. Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, Netherlands

P.27. Perceived Assistance Questionnaire is a useful tool for the self assessment of quality of care providers
Moreno JL, Redon P, Paipa AJ, Dominguez R, Turon J, Povedano M. ALS Unit, Hospital Universitari de Bellvitge, IDIBELL, Barcelona, Spain

P.29. Motor neuron disease with very long disease duration or CMT?
Giorgia Querin (1), Philippe Corcia (2,3), Pierre-Francois Pradat (2,4) 1) Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomédicale, Paris, France 2) CHRU Bretonneau, Service de Neurologie, Centre SLA, Tours, France. 3) INSERM U 930, Tours, France. 4) APHP, Hopital Pitié-Salpêtriere, Département des Maladies du Systéme Nerveux, Centre référent SLA, Paris, France

P.31. Longitudinal recordings of eye movements confirm sequential oculomotor alterations in ALS
Elmar H. Pinkhardt1, MD, Martin Gorges1, PhD, Hans-Peter Maller, PhD, Dorothée Lulé1, PhD, Kelly Del Tredici, MD, Jürgen Keller1, M.Sc., Albert C. Ludolph1, MD, Jan Kassubek1, MD, (Ulm) 1Department of Neurology, University of Ulm, Ulm, Germany 2Section Clinical Neuroanatomy, Department of Neurology, University of Ulm, Ulm, Germany

P.33. Longitudinal Effects of Mindfulness on People with Amyotrophic Lateral Sclerosis and their Caregiver
Francesco Pagnini, Psy.D., Ph.D. Department of Psychology, Università Cattolica del Sacro Cuore, Milan, Italy Department of Psychology, Harvard University, Cambridge, MA, USA

P.35. Increased risk of ALS for frontline workers
D'Ovidio F[1], d'Errico A[2], Calvo A[1], Costa G[2], Chiò A[1] [1] Rita Levi Montalcini Department of Neurosciences, University of Turin, Turin, Italy [2] Epidemiology Department ASL TO3 Piedmont Region, Grugliasco (TO), Italy

P.37. Hypothalamic dysfunctions in Amyotrophic Lateral Sclerosis
Pauline VERCRUYSSE (1,2), Jerame SINNIGER (1), Hajer EL OUSSINI (1), Jelena SCEKIC-ZAHIROVIC (1), Martin GORGES (2), Hans-Peter MULLER (2), Stephane DIETERLE (1), Sanaz GABERY (4), Dietmar THAL (3), Asa PETERSEN (4), Jan KASSUBEK (2), Anke WITTING (2), Albert LUDOLPH (2), Luc DUPUIS (1) and the GERP-ALS group. 1: INSERM U1118: Mécanismes centraux et périphériques de la neurodégénérescence, Faculté de médecine de Strasbourg, France 2: Department of Neurology, University of Ulm, Germany 3: Department of Neuropathology, University of Ulm, Germany 4: Translational Neuroendocrine Research Unit, Lund University, Lund, Sweden

P.39. Functional and structural disruption of transcallosal pathways in ALS: a DTI-TMS study
Bastian BöcklerÂ¹, Jan Kassubek[1], Jan Lewerenz[1], Ece Ercan[2], Alireeza Abei[3], Volker Rasche[3], Thomas Kammer, Itamar Ronen[3], Annemarie Hübers[1] 1 Department of Neurology, University Hospital of Ulm, Ulm, Germany 2 C. J. Gorter Center of High Field MRI, Department of Radiology, Leiden University Medical Center, Leiden, Netherlands 3 Experimental Cardiovascular MRI Group, Department of Internal Medicine II - Cardiology, University Hospital of Ulm, Ulm, Germany 4 Department of Psychiatry, University Hospital of Ulm, Ulm, Germany

P.41. Deep phenotyping of Frontotemporal Dementia (FTD) and FTD-MND in Ireland
Taha Omer*1&2, Siobhan Hutchinson2, Colin P Doherty2, Orla Hardiman1&2; 1-Academic Unit of Neurology, Trinity College Dublin, Ireland 2-St James Hospital Dublin, Ireland

P.43. Coexistence of ALS and CADASIL in an Albanian patient
C. Morelli1, A. Ciammola1, F. Girotti1, A. Lerario2,3, A. Doretti1,3, L. Pradotto4, M.Mencarelli5, A. Di Blasio5, A. Mauro4,6, L.Maderna1,3, V. Silani1,3. 1Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy 2UOD Malattie Neuromuscolari e Rare, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan Italy 3"Dino Ferrari" Center-Department of Pathophysiology and Transplantation-Università degli Studidi Milano, Milan, Italy 4Division of Neurology and Neurorehabilitation, IRCCS Istituto Auxologico Italiano, Milan, Italy 5Laboratory of Molecular Biology, IRCCS Istituto Auxologico Italiano, Milan, Italy 6Department of Neuroscience, University of Turin, Turin, Italy

P.45. CASE 1
Claudia Lazo La Torre, Luis Brieva, Cristina Mingot Multidisciplinary ALS Unit, University Hospital Arnau Vilanova, Catalonia – Spain, Gabinete de Pruebas Funcionales del Sistema Nervioso Neurofisiologia Clinica, Unidad de Neuromonitorización Intraoperatoria Unidad de ELA-MTN, Hospital Universitario Arnau Vilanova

P.47. BMAALS: is there a link between ALS and BMAA exposure?
Philippe Couratier,1,2, Farid Boumadine,1, William Camu,8,9, Emmeline Lagrange,7, Audrey Combs,4,5, Olivier Ploux,3, Valérie Pichon,4,5, Annick Méjean,3, Vincent Bonneterre,6, Gérard Besson,7, Marie Nicol,1,2, Auralie Delzor,1, Pierre-Marie Preux,1, Benoat Marin1 1 Tropical Neuroepidemiology, INSERM UMR 1094, Limoges, France 2 Department of Neurology, ALS Center, University Hospital Dupuytren, Limoges, France 3 Interdisciplinary Laboratory for Tomorrowâs Energy Pack (LIED), CNRS UMR 8236, University Paris Diderot-Paris 7, Paris, France 4 Department of Analytical, Bioanalytical Sciences and Miniaturization (LSABM), UMR ESPCI-ParisTech-CNRS 8231 CBI, Paris, France 5 University Sorbonne, University Pierre and Marie Curie (UPMC), Paris, France 6 Environment and Health Prediction in Populations (EPSP), CNRS-TIMC-IMAG UMR 5525 UJF-Grenoble 1, Grenoble, France 7 Department of Neurology, University Hospital of Grenoble, Grenoble, France 8 Motoneuron Diseases: Neuroinflammation and Therapy, INSERM UMR 1051, Neurosciences Institute, Montpellier, France 9 Department of Neurology, ALS Center, University Hospital Gui de Chauliac, Montpellier, France

P.49. Artificial Neural Networks in Forecasting ALS progression from Clinical Data
Emanuele Borgonovo, Bocconi University, Milan

P.51. ALS Centre Moscow: 4 years' experience
Brylev L., Shtabnitskiy V., Parshikov V., Vorobyeva A., Lysogorskaya E., Ivanova M., Dikhter E., Sergeeva S., Ataulina A., Zakharova M. ALS Centre Moscow

P.53. A population-based case-control study to assess sleep disturbances as a risk factor for ALS
Anne E Visser1, Jeroen W Bos1, Henk-Jan Westeneng1, Oscar J Vogels2, Jan H Veldink1, Leonard H van den Berg1 1 Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands 2 Department of Neurology, St. Antonius Hospital, Nieuwegein, The Netherlands

P.55. Perception of dignity in ALS patients and its influence on emotional distress
Amparo Martinez1,2, David Rudilla3, Juan F Vázquez-Costa1,4,5. 1 ALS Unit, Department of Neurology, Hospital Universitario y Politècnico La Fe, Valencia, Spain. 2 Carena Association. Psychological support in Cancer. 3 HHS. Hospital at home services 4Neuromuscular Research Unit, Instituto de Investigacion Sanitaria la Fe (IIS La Fe), Valencia, Spain. 5Centro de Investigacion Biomédica en Red de Enfermedades Raras (CIBERER), Spain. 5 ALS Unit, Department of Rehabilitation, Hospital Universitario y Politècnico La Fe, Valencia, Spain.

Cognitive/Behaviour I (P57-P69)

P.57. The validation of the Italian Edinburgh Cognitive and Behavioural ALS Screen (ECAS)
Barbara Poletti1, Federica Solca1, Laura Carelli1, Fabiana Madotto2, Annalisa Lafronza1, Andrea Faini3, Alessia Monti4, Stefano Zago5, Daniela Calini1, Cinzia Tiloca1, Alberto Doretti1,6, Federico Verde1,6, Antonia Ratti1,6, Nicola Ticozzi1,6, Sharon Abrahams7, Vincenzo Silani1,6 1 Department of Neurology and Laboratory of Neuroscience - IRCCS Istituto Auxologico Italiano, Milan, Italy 2 Research Centre on Public Health. Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy 3 Department of Cardiovascular, Neural and Metabolic Sciences - IRCCS Istituto Auxologico Italiano, Milan, Italy 4 Department of Neurorehabilitation Sciences, Casa Cura Policlinico, Milan, Italy 5 Department of Neuroscience and Mental Health, Università degli Studidi Milano, IRCCS Fondazione Caâ' Granda Ospedale Maggiore Policlinico, Milan, Italy. 6 Department of Pathophysiology and Transplantation, Dino Ferrariâ Center, Università degli Studidi Milano, Milan, Italy 7 Euan MacDonald Centre for Motor Neuron Disease Research, Anne Rowling Regenerate Neurology Clinic, Centre for Cognitive Ageing and Cognitive Epidemiology, Human Cognitive Neuroscience-PPLS, University of Edinburgh, Edinburgh, UK

P.59. Standardization and Validation of the ECAS using Age and Education Adjusted Norms
Marta Pinto-Grau M.Psych(ClinNeuroPsych) 1,2, Tom Burke M.Psych.Sc 1,2, Katie Lonergan BA 1,2, Caroline McHugh MSc 3, Iain Mays MSc 1, Caoifa Madden MSc 1, Alice Vajda PhD 1, Mark Heverin MSc 1, Marwa Elamin PhD 1, Orla Hardiman MD FRCPI FTCD MRIA 1, Niall Pender PhD 1,2. 1. Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland. 2. Department of Psychology, Beaumont Hospital, Dublin, Ireland. 3. University of Surrey, Guildford, UK

P.61. Psychophysiological principles underlying pathological laughing and crying in ALS
Annemarie Hübers[1], Jan Kassubek[1], Georg Grün[2], Martin Gorges[1], Helena Aho-Oezhan[1], Jürgen Keller[1], Hannah Horn[1], Hermann Neugebauer[1], Ingo Uttner[1],

Dorothee Lulé[1], Albert C. Ludolph[1]
1 Department of Neurology, University Hospital of Ulm, 89081 Ulm, Germany 2 Department of Psychiatry, University Hospital of Ulm, 89073 Ulm, Germany

P.63. New frontiers of cognitive assessment in neurodegenerative disease: proof of concept by means of ET
Barbara Poletti1*, Laura Carelli1, Federica Solca1, Annalisa Lafronza1, Elisa Pedroli2, Andrea Faini3, Stefano Zago4, Nicola Ticozzi1,5, Paolo Meriggi6, Pietro Cipresso2, Dorothee Lulé8, Albert C. Ludolph8, Giuseppe Riva2-7, Vincenzo Silani1,5 1 Department of Neurology and Laboratory of Neuroscience - IRCCS Istituto Auxologico Italiano, Milan, Italy 2 Applied Technology for Neuro-Psychology Lab, IRCCS Istituto Auxologico Italiano, Milan, Italy 3 Department of Cardiovascular, Neural and Metabolic Sciences - IRCCS Istituto Auxologico Italiano, Milan, Italy 4 Department of Neuroscience and Mental Health, Università degli Studidi Milano, IRCCS Fondazione Caâ' Granda Ospedale Maggiore Policlinico, Milan, Italy. 5 Department of Pathophysiology and Transplantation, Dino Ferrariâ Center, Università degli Studidi Milano, Milan, Italy 6 ICT & Biomedical Technology Integration Unit, Centre for Innovation and Technology Transfer (CITT), Fondazione Don Carlo Gnocchi Onlus, Milan, Italy 7 Department of Psychology, Università Cattolica del Sacro Cuore, Milan, Italy 8 Department of Neurology - University of Ulm, Ulm, Germany

P.65. Depression in amyotrophic lateral sclerosis
E. Roos (a), D. Mariosa (a), C. Ingre (b), C. Lundholm (a), K. Wirdefeldt (a,b), P. M. Roos (c,d), F. Fang (a) (a) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (b) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden (c) Department of Neurology, Division of Clinical Neurophysiology, Oslo University Hospital, Oslo, Norway (d) Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

P.67. Cognitive assessment in ALS by means of P300-Brain Computer Interface: a preliminary study
Barbara Poletti1, Laura Carelli1, Federica Solca1, Annalisa Lafronza1, Elisa Pedroli2, Andrea Faini3, Stefano Zago4, Nicola Ticozzi1,5, Paolo Meriggi6, Pietro Cipresso2-7, Dorothee Lule8, Albert C. Ludolph8, Giuseppe Riva2-7, Vincenzo Silani1,5 1 Department of Neurology and Laboratory of Neuroscience - IRCCS Istituto Auxologico Italiano, Milan, Italy 2 Applied Technology for Neuro-Psychology Lab, IRCCS Istituto Auxologico Italiano, Milan, Italy 3 Department of Cardiovascular, Neural and Metabolic Sciences - IRCCS Istituto Auxologico Italiano, Milan, Italy 4 Department of Neuroscience and Mental Health, Università degli Studidi Milano, IRCCS Fondazione Caâ' Granda Ospedale Maggiore Policlinico, Milan, Italy. 5 Department of Pathophysiology and Transplantation, Dino Ferrariâ Center, Università degli Studidi Milano, Milan, Italy 6 ICT & Biomedical Technology Integration Unit, Centre for Innovation and Technology Transfer (CITT), Fondazione Don Carlo Gnocchi Onlus, Milan, Italy 7 Department of Psychology, Catholic University of Milan, Milan, Italy 8 Department of Neurology - University of Ulm, Ulm, Germany

P.69. Reliable Change Index (RCI) as metric for significant change in cognitively intact ALS patients
Tom Burke 1,2, Marta Pinto-Grau 1,2, Katie Lonergan 1,2, Orla Hardiman, 2,3, & Niall Pender 1,2. 1 Department of Psychology, Beaumont Hospital. 2 Academic Unit of

Neurology, Trinity Biomedical Sciences Institute. 3 Department of Neurology, Beaumont Hospital.

Genes and Genomics I (P71-P85)

P.71. Whole exome sequencing reveals novel and known rare FIG4 mutations in a central European ALS cohort
Alma Osmanovic 1,2, Isolde Rangnau 1,2, Anne Kosfeld 2, Susanne Abdulla 1, Claas Janssen 1, Ruthild G. Weber 2, Susanne Petri 1 1 Department of Neurology, Hannover Medical School, Hannover, Germany 2 Department of Human Genetics, Hannover Medical School, Hannover, Germany

P.73. Transcriptome analysis in a motor neuron muscle microfluidics system in ALS
Jik Nijssen1, Julio Cesar Aguila1,2, Laura Comley1, Qiaolin Deng2 and Eva Hedlund1 1. Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden 2. Department of Cell and Molecular Biology, Karolinska Institutet, 171 77 Stockholm, Sweden

P.75. Project MinE: Study design and pilot analyses of a large-scale whole genome sequencing study in ALS
Project MinE Consortium

P.77. Molecular diagnosis of Amyotrophic Lateral Sclerosis by Next Generation Sequencing (NGS) analysis of a French cohort of patients
Marouillat S1*, Brulard C1*, ,Thapault RA1, Antar C2, Maurel C1, Dangoumau A1, Blasco H1,2, Andres CR1,2, Vourc'h P1,2, Corcia P3 1 UMR INSERM U930, Università Francois Rabelais, 37032 Tours, France * Co-authors 2 CHRU de Tours, Service de Biochimie et de Biologie Moleculaire, 37044 Tours, France 3 CHRU de Tours, Centre de Ressources et de Compétence SLA, 37044 Tours, France

P.79. Genetic Background of C9orf72 Expansion and Epigenetic Modifications in Turkish ALS Cases
Ceren Tunca, Fulya Akcimen, Hamid Hamzeiy, A. Nazli Basak Department of Molecular Biology and Genetics, NDAL, Bogazici University, Istanbul, Turkey

P.81. Causes and consequences of microRNA dysregulation in ALS
Eran Hornstein, Weizmann Institute of Science, Israel

P.83. A target NGS approach to clarify the role of genetic variants in ALS pathogenesis
Giuseppe Marangi (1), Serena Lattante (1), Paolo Niccolò² Doronzio (1), Amelia Conte (2), Katia Patanella (2), Marcella Zollino (1), Mario Sabatelli (2) (1) Institute of Genomic Medicine, Università Cattolica del Sacro Cuore, Roma (2) NEMO Clinical Centre-Roma, Institute of Neurology, Università Cattolica del Sacro Cuore, Roma

P.85. A novel p.Leu106fs*15 SOD1 mutation with absence of the mutated protein: a case report
Canosa A, Barberis M, Calvo A, Rinaudo MT, Lomartire A, Brunetti M, Marrali G, Casale F, Fuda G, Salamone P, Solero L, Di Cunto F, Turco E, Mora G, De Marco G, Chiò A ALS Center of Turin, "Rita Levi Montalcini" Department of Neuroscience, University of Turin, Turin, Italy (Canosa A, Barberis M, Calvo A, Lomartire A, Brunetti M, Marrali G, Casale F, Fuda G, Salamone P, Solero L, De Marco G, Chiò A). Department of Oncology, University of Turin, Turin, Italy (Rinaudo MT). Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy (Di Cunto F). Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy (Turco E). Department of Neurological Rehabilitation, Fondazione Salvatore Maugeri, IRCCS, Istituto Scientifico di Milano, Milan, Italy (Mora G)

Mechanisms of Disease I (P87-P109)

P.87. UNEXPECTED ROLE OF NUCLEAR SUPEROXIDE DISMUTASE 1
Bordoni M.1,2 Pansarasa O.1, Crippa V.1, Dell'Orco M.1,Gagliardi S.1, Diamanti L.3,4, Poletti A.5, Ceroni M.3,4, Cereda C.1 1Laboratory of Experimental Neurobiology, C. Mondino National Institute of Neurology Foundation, IRCCS, via Mondino 2, 27100 Pavia, Italy. 2Department of Biology and Biotechnology L. Spallanzani, University of Pavia, Pavia, Italy. 3Departement of Brain and Behavioural Science, University of Pavia, Pavia, Italy. 4General Neurology Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia, Italy. 5Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studidi Milano, Milano, Italy

P.89. TGF 1 effects in ALS muscle and motorneuron
Meroni M.,Cicardi M.E.,Cristofani R.,Crippa V.,Rusmini P.,Messi E.,Galbiati M.,Poletti A. Dipartimento di Scienze Farmacologiche e Biomolecolari-Centre of Excellence on Neurodegenerative Diseases, Università degli Studidi Milano, Italia. Inter-University Research Centre on the Molecular Basis of Neurodegenerative diseases (Universities of Florence, Rome and Milan, Italy)

P.91. TDP-43 sequestration and aggregation reflects its loss of function also at the proteome level
Sonja Prpar Mihevc1, Emanuele Buratti2, Marco Baralle2, Federico E. Baralle2, Boris Rogelj1,3 1 Department of Biotechnology, Jozef Stefan Institute, Jamova 39, Ljubljana, Slovenia 2 ICGEB International Centre for Genetic Engineering and Biotechnology, Trieste, Italy 3 Biomedical Research Institute BRIS, Ljubljana, Slovenia

P.93. TDP-43 and FUS are exported from the nucleus independent of Exportin-1/CRM1
Helena Ederle and Dorothee Dormann Affiliation: Biomedical Center (BMC), Institute for Cell Biology (Anatomy III), Ludwig Maximilians University Munich, GroÃŸhaderner Strasse 9, 82152 Planegg Martinsried

P.95. Novel cellular models of FUS pathology created by targeted modification of the FUS gene
Haiyan An, School of Biosciences, Cardiff University, UK. Tatyana Shelkovnikova, School of Biosciences, Cardiff University, UK. Vladimir Buchman, School of Biosciences, Cardiff University, UK

P.97. Neuregulin and ErbB4 receptor abnormalities in Amyotrophic Lateral Sclerosis
Mireia Herrando-Grabulosa1, Belan Garcia2, Renzo Mancuso3, Anna Martinez, Guillem Madol1, Assumpcia³ Bosch2 and Xavier Navarro1 1Department of Cell Biology, Physiology and Immunology, Institute of Neurosciences and CIBERNED, Universitat Autonoma de Barcelona, Bellaterra, Spain.2 Center of Animal Biotechnology and Gene Therapy and Department of Biochemistry and Molecular Biology, Universitat Autonoma de Barcelona , Bellaterra, Spain.3Centre for Biological Sciences, University of Southampton, Southampton, UK

P.99. MIF alters misfolded SOD1 amyloid aggregation by inducing the formation of disordered aggregates
Tom Shani1, Guy Zoltsman1, Victor Banerjee2, Joy Kahn1, Salah Abu-Hamad1, Maria Vyazmensky4,5, Daniel Gitler1,3, Niv Papo2,5, Stanislav Engel4,5 and Adrian Israelson1 1Department of Physiology and Cell Biology, Faculty of Health Sciences, 2Department of Biotechnology Engineering, Faculty of Engineering, 3The Zlotowski Center for Neuroscience, 4Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, 5The National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, P.O.B. 653 Beer Sheva, 84105, Israel

P.101. Enhancing mitochondrial fusion is neuroprotective in TDP-43 Drosophila models of ALS
Bilal Khalil1, Marie-Jeanne Cabirol-Pol1, Alexander J Whitworth2, Magalie Lecourtois3,4 and Jean-Charles Liavens1 1Aix-Marseille Université, CNRS, Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, UMR 7286, 13344 Marseille, France 2MRC Mitochondrial Biology Unit, Cambridge Biomedical Campus, Cambridge, CB22 LY, United Kingdom 3Inserm, U1079, 76183 Rouen, France 4IRIB Institute for Research and Innovation in Biomedicine, University of Rouen, 76183 Rouen, France

P.103. Clearance and transport of misfolded protein responsible for motor neuron diseases (MNDs)
Cristofani R. (1,4),Crippa V. (2), Cicardi M.E.(1,4), Rusmini P.(1,4), Meroni M. (1,4), Vezzoli G. (1,4), Ferrari V. (1,4), Licata N.V. (1,4), Galbiati M. (1,4), Carra S.(3), Poletti A.(1,4) 1. Sezione di Biomedicina ed Endocrinologia, Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studidi Milano, Milano, Italy. 2. Laboratory of Experimental Neurobiology, Genomic and post-Genomic Center, C. Mondino National Neurological Institute, Pavia, Italy 3. Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università degli Studidi Modena e Reggio Emilia, Modena, Italy 4. Centro InterUniversitario sulle Malattie Neurodegenerative, Università degli Studidi Firenze, Genova, Roma Tor Vergata and Milano, Italy

P.105. Characterization of TDP-43 splicing target TNIK in neuronal differentiation
Valentina Gumina1,2, Claudia Colombrita1,3, AnnaMaria Maraschi1, Emanuele Buratti4, Francisco E. Baralle4, Vincenzo Silani1,3, Antonia Ratti1,3 1Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Milan, Italy. 2Doctorate School of Molecular Medicine, Università degli Studidi Milano, Milan, Italy. 3Department of Pathophysiology and Transplantation, 'Dino Ferrari' Center - Università degli Studidi Milano, Milan, Italy. 4International Centre for Genetic Engineering and Biotechnology (ICGEB), AREA Science Park, Trieste, Italy

P.107. Calcium-responsive transactivator protein (CREST) shares common properties with other ALS-associated proteins
Michail S Kukharsky1,2, Annamaria Quintiero1, Taisei Matsumoto3, Koji Matsukawa3, Haiyan An1, Tadafumi Hashimoto3, Takeshi Iwatsubo3, Vladimir L Buchman1 and Tatyana A Shelkovnikova1,2 1School of Biosciences, Cardiff University, Museum Avenue, CF10 3AX, Cardiff, UK. 2Institute of Physiologically Active Compounds Russian Academy of Sciences, 1 Severniy proezd, Chernogolovka, 142432, Moscow Region, Russian Federation 3Department of Neuropathology, The University of Tokyo, Tokyo, Japan

P.109. Analysis of the hnRNP A family in health and disease in zebrafish
Lara Jansen 1 Christian Haass 1,2,3 Bettina Schmid 1,3 1: German Center for Neurodegenerative Diseases, Munich, Germany 2: Munich Cluster for Systems Neurology (Synergy), Munich, Germany 3: Biomedical Center (BMC), Ludwig Maximilians University, Munich, Germany

Therapy I (P111-P135)

P.111. The CANALS study: A RCT to Assess Safety and Efficacy on Spasticity of a C. Sativa Extract in MND
Nilo Riva,1 Gabriele Mora,2 Gianni Sorarù[1],3 Christian Lunetta,4 Marta Clerici,1 Yuri Falzone,1 KalliopiMarinou,2 Eleonora Maestri,4 Raffaella Fazio,1 Mauro Comola,1 Giancarlo Comi1, The CANALS study Group 1Department of Neurology, Division of Neuroscience, Institute of Experimental Neurology (INSPE), Ospedale San Raffaele Scientific Institute, Milan, Italy, 2Neurologic Department - Fondazione Salvatore Maugeri IRCCS, Istituto Scientifico di Milano, Milan, Italy, 3Department of Neurosciences, University of Padova, Padova, Italy, 4NEuroMuscular Omnicentre (NEMO), Fondazione Serena Ospedale granda, Milan, Italy

P.113. SOD1/Rag2-/- mice with a low copy number of the SOD1 gene as a model for stem cell therapy of ALS
Luiza Stanaszek1, Malgorzata Majchrzak1, Anna Jablonska2, Ophelia Rogers2, Sujith Sajja2, Barbara Lukomska1, Piotr Walczak2, Miroslaw Janowski1,2 1Mossakowski Medical Research Centre PAS, NeuroRepair Department 2Johns Hopkins University School of Medicine, Institute for Cell Engineering, Division of MR Research, The Russell H. Morgan Department of Radiology and Radiological Science

P.115. Riluzole display glutamate-independent antioxidant properties: relevance for ALS
Lucio Tremolizzo, Alessandro Arosio, Gessica Sala, Elisa Conti, Simone Beretta, Carlo Ferrarese Lab. of Neurobiology, School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza, Italy

P.117. Targeting MCU within ERMCC in ALS
Tadic V1 and Goldhammer N1, Malci A1, Slesiona N1, Stubendorff B1, Prell T1, Liu J 1, Witte OW1,2, Grosskreutz J1 1 Hans Berger Department of Neurology, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany 2 Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena

P.119. Pharmacological manipulation of Sig1R affects ER-mitochondrial interplay in G93A model of ALS
Tadic V 1, Malci A 1, Goldhammer N1, EM Piskor 1, Stubendorff B1, Prell T 1, Liu J 1, Witte OW1,2, Grosskreutz J 1 1 Hans Berger Department of Neurology, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany 2 Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena

P.121. Macrophage Migration Inhibitory Factor as a Modifier of Mutant SOD1 Toxicity in a Mouse Model of ALS
Marcel F. Leyton, Clara Benaim, Amos Guetta, Salah Abu-Hamad and Adrian Israelson Department of Physiology and Cell Biology, Faculty of Health Sciences and The Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, P.O.B. 653 Beer Sheva, 84105, Israel

P.123. Intraspinal injection of human mesenchymal stromal cells in SOD1G93A ALS mice
Bursch F 1, 2, Rath KJ 1, Sarikidi A1, Baselt S.1, Petri S.1, 2 1 Department of Neurology, Hannover Medical School; 2 Centre for Systems Neuroscience (ZSN) Hannover, Germany

P.125. Impaired exercise tolerance in ALS is related to reduced oxidative metabolism of skeletal muscles
L. Tremolizzo,1,3 A. Ferri,1,2 G. Corna,1 A. Bosio,1,3 C. Lunetta,4 V. Silani,5 N. Riva,6 A. Rigamonti,7 A. Maggiani,8 C. Ferrarese,1,3 F. Lanfranconi,1 for the ME and ALS study group* 1School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), Univ. of Milano-Bicocca, Italy; 2Clinical Exercise Science Research Program, Institute of Sport, Exercise and Active Living (ISEAL), Victoria Univ., Melbourne, Australia; 3Neurology Unit, San Gerardo Hospital, Monza, Italy; 4NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milano, Italy; 5IRCCS Istituto Auxologico Italiano, Univ. degli Studi di Milano, Italy; 6San Raffaele Hospital, Milano, Italy; 7Alessandro Manzoni Hospital, Lecco, Italy; 8Italian Academy of Osteopathic Medicine (AIMO), Saronno, Italy; *collaborators are listed separately. *the ME and ALS study group, besides the Authors includes: Riccardo Bonazzi (Monza), Dario Bovio (Milano), Diletta Cereda (Monza), Andrea Della Valentina (Saronno), Marco Grandini (Saronno), Carolina Lavazza (Saronno), Andrea Magnoni (Monza), Laurent Mapelli (Saronno), Vittorio Mantero (Lecco), Samuele Marchese (Saronno), Ornella Mauri (Monza), Valeria Milano (Saronno), Paola Prometti (Monza), Caterina Salito (Milano), Andrea Salmaggi (Lecco), Silvia Sosio (Saronno), Barbara Uva (Milano)

P.127. Impact of ifnγ on neurotoxicity and er-mitochondria coupling cycle in ALS
Sengupta S1, Tadic V1, Malci A1, Liu J1, Le TT1, Stubendorff B1, Witte OW1,2, Prell T1*, Grosskreutz J1* * shared last authorship 1 Hans Berger Department of Neurology, Jena University Hospital, Erlanger Allee 101, 07747 Jena, Germany 2 Center for Sepsis Control and Care, Jena University Hospital, Erlanger Allee 101, 07747 Jena

P.129. Effect of CDNF administration in SOD1-G93A mouse model of Amyotrophic Lateral Sclerosis
Voutilainen MH 1., De Lorenzo F 1., Montonen E 1., Saukkonen A 1., Airavaara M 1., Tuominen RK 2., Lindholm D 3., Sendtner M 4., Saarma M1. Institute of Biotechnology, University of Helsinki, Finland 1, Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki 2, Institute of Biomedicine, University of Helsinki 3,. Institute of Clinical Neurobiology, University of Wuerzburg 4, Germany

P.131. Developing vertebrate models to highlight the relevance of Nefl and miRNAs in ALS pathogenesis
Doris Lou Demy, Raphael Munoz and Edor Kabashi, Institut du Cerveau et de la Moelle Epinière, Hopital Pitié-Salpêtrière, Paris, France

P.133. Additive effects of HGF, Artemin and CNTF on ALS-relevant motor neurons isolated by high speed FACS
Sébastien Schaller1, Dorothée Buttigieg1, Arnaud Jacquier1, David Gentien2, Pierre Delagrange3, Mark Merchant4, Marc Barad5, Georg Haase1 1 Institut de Neurosciences de la Timone, UMR 7289 CNRS & Aix-Marseille University, 27 bd Jean Moulin, 13005 Marseille, France. 2 Institut Curie, Plateforme Affymetrix de biologie moleculaire, 26 Rue d'Ulm, 75005 Paris, France. 3 GenoSplice technology, iPEPS - ICM, Hopital Pitié Salpêtrière, 47/83, bd de l'Hopital, 75013 Paris, France. 4 Genentech Inc, 1 DNA Way, M/S 245c, South San Francisco, CA 94080, USA. 5 Centre d'Immunologie de Marseille-Luminy, Parc Scientifique & Technologique de Luminy, case 906, 13288 Marseille, France

P.135. A preliminary study of chaperone-mediated autophagy in ALS lymphomonocytes
Lucio Tremolizzo,1,2 Alessandro Arosio,1 Riccardo Cristofani,3 Gessica Sala,1 Valeria Crippa,4 Christian Lunetta,5 Angelo Poletti,3 Carlo Ferrarese,1,2 1School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), Univ. of Milano-Bicocca; 2Dep. of Neurology, San Gerardo Hospital, Monza; 3Dip. di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Univ. degli Studi di Milano; 4Lab. di Neurobiologia Sperimentale, Istituto Neurologico Nazionale C. Mondino, Pavia; 5NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milan; Italy.

Poster Session II (Even number Abstracts)

Friday 20th May, h.17.30-19.00

Biomarkers II (P2-P12)

P.02. Serum C-reactive protein (CRP) as a simple and independent prognostic biomarker in ALS
Christian Lunetta, MD1, Andrea Lizio1, Eleonora Maestri1, Gabriele Mora, MD2, Andrea Calvo, MD3, Adriano Chiò, MD, FAAN3,4 1 NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus , Milano, Italy, 2 Department of Neurological Rehabilitation, Fondazione Salvatore Maugeri, IRCCS, Istituto Scientifico di Milano, Milano, Italy. 3 ALS Center, ˜Rita Levi Montalcini Department of Neuroscience, Neurology II, University of Torino 4 Azienda Ospedaliero-Universitaria Citta della Salute e della Scienza, Torino, Italy

P.04. Mutated and non-mutated amyotrophic lateral sclerosis: a neurophysiological comparison
Ungaro D*, Doretti A.*, Ticozzi N*, Ratti A^, Tiloca C^, Gregorini F*, Riccardi B*, Silani V*, Maderna L* * Department of Neurology and Neurophysiology, Istituto Auxologico Italiano, Milan , Italy ^ Laboratory of Neuroscience - IRCCS Istituto Auxologico Italiano Milan, Italy

P.06. Metal and proteomic analysis of sporadic ALS patients with common geographical origin
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P.08. Early- and late-onset biomarkers in peripheral blood mononuclear cells of ALS patients
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P.10. Circulating exosomes as a novel source of biomarkers for ALS progression
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P.12. Alterations of Adiponectin in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia
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Clinical aspects II (P14-P56)

P.14. The selective anatomical vulnerability of ALS – disease defining & disease defying brain regions
Peter Bede - Trinity College Dublin, Ireland

P.16. The ALS Stratification Challenge- Using big data to develop models for stratifying ALS patients
Maya Bronfeld 1, Robert Kueffner 2, Nazem Atassi 3, VenkatBalagurusamy 4, Barbara di Camillo 5, Merit Cudkowicz 3, Donna Dillenberger 4,Javier Garcia-Garcia 6, Orla Hardiman 7, Bruce Hoff 8, Joshua Knight 4, Melanie Leitner 9, Guang Li 10, Lara Mangravite 8, Raquel Norel 4, Thea Norman 8, Liuxia Wang 10, Gustavo Stolovitzky 4, Neta Zach 1 1 Prize4Life, Israel 2 Ludwig-Maximilian-University, Germany 3 Massachusetts General Hospital, MA, USA 4 IBM Research, NY, USA 5 University of Padova, Italy 6 Pompeu Fabra University, Spain 7 Trinity College Institute of Neuroscience, Ireland 8 Sage Bionetworks 9 Biogen Idec, MA, USA 10 Origent, VA, USA

P.18. Survival of ALS patients is not affected by a psychosocial intervention in an ALS unit
Paipa AJ, Verges E, Dominguez R, Turon J, Povedano M. ALS Unit Hospital de Bellvitge, IDIBELL, Barcelona, Spain Fundacio Miquell Valls

P.20. Sialorrheia and reversals in ALSFRS
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P.22. Ptosis and bulbar-onset in familial ALS: two different diseases or a new clinical phenotype?
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P.24. Predictors of adaptation to non-invasive ventilation in Amyotrophic Lateral Sclerosis
Russo M1, Bonanno C2, Profazio C1, La Foresta S1, Faraone C1, Lizio A3, Vita GL1, Sframeli M1,2, Di Stefano MG2, La Rosa M2, Barcellona C2, Ruggeri P 2,Vita G1,2, Lunetta C1,3, Messina S1,2 1 Nemo Sud Clinical Center for Neuromuscular Disorders, University Hospital G. Martino, Messina, Italy 2 Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy 3 NEuroMuscular Omnicenter, Serena Onlus Foundation, Milan, Italy

P.26. Phenotype comparison between young- and elderly-onset motor neuron disease patients
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P.28. PLS-FTLD, expanding the spectrum of dementia in motor neuron disease
Balint S. de Vries 1, Laura Rustemeijer 1, Anneke van der Kooi 2, Joost Raaphorst 2,3, Carin D. Schröder 4, Tanja Nijboer 4,5, Jeroen Hendrikse 6, Jan H. Veldink 1, Leonard H. van den Berg 1 & Michael A. van Es 1 1 Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands. 2 Department of Neurology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. 3 Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands. 4 Brain Center Rudolf Magnus and Center of Excellence for Rehabilitation Medicine, University Medical Center Utrecht and De Hoogstraat Rehabilitation, Utrecht, The Netherlands. 5 Department of Experimental Psychology, Helmholtz Institute, Utrecht University, Utrecht, The Netherlands. 6 Department of Radiology, University Medical Center Utrecht, the Netherlands

P.30. Motor Unit Number index (MUNIX) of six muscles: Normal values and effects of age and gender
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P.32. Longitudinal characteristics of latent ALSFRS-R subscores
James Rooney(1), Tom Burke(1), Alice Vajda(1), Mark Heverin(1), Orla Hardiman(1,2) 1. Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland 2. Neurology Department, Beaumont Hospital, Dublin, Ireland

P.34. Is firstly diagnosed ALS really ALS? Results of a population-based study with long-term follow-up
Elisabetta Pupillo, Ettore Beghi and the SLALOM group. IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

P.36. Impairment of sensory-motor integration at spinal level in amyotrophic lateral sclerosis
Veronique MARCHAND-PAUVERT Inserm U146, France

P.38. Gray matter changes and pseudobulbar affect in patients with ALS: a voxel-based morphometry study
Foteini Christidi1, Efstratios Karavasilis2, Panagiotis Ferentinos3, Michael Rentzos1, Sophia Xirou1, Vasiliki Zouvelou1, Georgios Velonakis2, Loukia S. Poulou2, Panagiotis Toulas2, Ioannis Zalonis1, Thomas Zambelis1, Nikolaos Karandreas1, Nikolaos Kelekis2, Ioannis Evdokimidis1 1 First Department of Neurology, Aeginition Hospital, Medical School, National & Kapodistrian University, Athens, Greece 2 Second Department of Radiology, University General Hospital Attikon, Medical School, National & Kapodistrian University, Athens, Greece 3 Second Department of Psychiatry, University General Hospital AttikonMedical School, National & Kapodistrian University, Athens, Greece

P.40. Euro-MOTOR: A case-control study of hormonal exposures as risk factors for ALS in women
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P.42. Critical issues in the Euro-MOTOR case-control study
D'Ovidio F[1], Rooney JPK[2], Visser AE[3], Vermeulen RCH[4], Veldink JH[3], Van den Berg LH[3], Hardiman O[2], Beghi E[5], Logroscino G[6], Chiò A[1] for the EUROMOTOR Group. [1] Rita Levi Montalcini Department of Neurosciences, University of Turin, Turin, Italy [2] Academic Unit of Neurology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland [3] Department of Neurology, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, the Netherlands. [4] Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands [5] Laboratorio di Malattie Neurologiche, Dipartimento di Neuroscienze, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy. [6] Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari 'Aldo Moro', Bari, Italy

P.44. Clinical epidemiology of amyotrophic lateral sclerosis in liguria, italy
Carlo Scialò[2], MD, Giovanni Novi, MD, Monica Bandettini Di Poggio, MD, Antonio Canosa, MD, Maria Pia Sormani, MD, Paola Mandich, MD, Paola Origone, MD, Romina

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P.46. Brain computer interface with P300-Speller: Feasibility of use for disabled ALS patients
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P.48. Big data, collaborations and patient centricity in ALS
Alex Sherman, Massachusetts General Hospital and Harvard Medical School, USA

P.50. ALS outcome measures and the role of smoke and vascular risk factors: a population-based study
Cristina Moglia; Andrea Calvo; Antonio Canosa; Paolo Cugnasco; Luca Solero; Umberto Manera; Marinella Clerico,; Enrica Bersano; Letizia Mazzini; Adriano Chiò ALS Center, Rita Levi Montalcini Department of Neuroscience, University of Turin, Turin, Italy (Chiò, Calvo, Canosa, Bertuzzo, Cugnasco, Solero, Manera, Moglia); Department of Biological and Clinical Science, University of Turin, and Azienda Ospedaliero Universitaria San Luigi Gonzaga, Orbassano (TO), Italy (Clerico, De Mercanti); ALS Center, Department of Neurology, Azienda Ospedaliero Universitaria Maggiore della Carita , Novara, Italy (Bersano, Mazzini); Salvatore Maugeri Foundation, IRCSS, Scientific Institute of Milano, Milano Italy (Marinou, Mora); Department of Neurology, Azienda Ospedaliera Regionale di Aosta , Azienda USL Valle d'Aosta, Aosta, Italy (Bottacchi); Salvatore Maugeri Foundation, IRCSS, Scientific Institute of Veruno (NO), Italy (Pisano)

P.52. Acoustic reflex patterns in amyotrophic lateral sclerosis
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P.54. A case of late-onset Obsessive Compulsive Disorder developing Upper MND and Frontotemporal Dementia
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P.56. Insights into Amyotrophic Lateral Sclerosis from a Machine Learning Perspective
Jonathan Gordon, Boaz Lerner Ben-Gurion University of the Negev, Be'er Sheva, Israel Department of Industrial Engineering and Management

Cognitive/Behaviour II (P58-P68)

P.58. The relationship between Apathy and Executive Dysfunction in ALS
Ratko Radakovic 1,2,3,4,5 , Laura Stephenson 2, Judith Newton 2, Christopher Crockford 1,4, Robert Swingler 2,4, Siddharthan Chandran 2 and Sharon Abrahams 1,2,4,5 1 Psychology- School of Philosophy, Psychology & Language Sciences, University of Edinburgh, Edinburgh, UK 2 Anne Rowling Regenerative Neurology Clinic, University of Edinburgh, Edinburgh, UK 3 Alzheimer Scotland Dementia Research Centre, University of Edinburgh, Edinburgh, UK 4 Euan MacDonald Centre for MND Research, University of Edinburgh, Edinburgh, UK 5 Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK

P.60. Slovenian version of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) - preliminary results
Petra Prunk[1], Ana Å tublar[1], Vita Å tukovnik[2], David Gosar[3], Janez Zidar[1], Leja Dolenc Groaelj[1], Lea Leonardis[1], Blaz Koritnik[1] [1]Ljubljana ALS Centre, Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia [2]Unit for Neurorehabilitation, Department of Neurology, University Medical Centre Ljubljana, Slovenia Â[3]Department of Pediatric Neurology, University Medical Centre Ljubljana, Slovenia

P.62. Psychological status and emotional burden in ALS caregivers: the role of metacognitive processes
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P.64. Executive and non-executive cognitive changes and pseudobulbar affect in ALS
Foteini Christidi1*, Panagiotis Ferentinos2*, Ioannis Zalonis1, Michael Rentzos1, Sophia Xirou1, Vasiliki Zouvelou1, Efstratios Karavasilis3, Thomas Zambelis1, Nikolaos Karandreas1, Ioannis Evdokimidis1 1 First Department of Neurology, Aeginition Hospital, Medical School, National & Kapodistrian University, Athens, Greece 2 Second Department of Psychiatry, University General Hospital Attikon, Medical School, National & Kapodistrian University, Athens, Greece 3 Second Department of Radiology, University General Hospital Attikon, Medical School, National & Kapodistrian University, Athens, Greece *shared first-authorship

P.66. Cognitive impairments may affect general decisional capacity, not personal therapeutic decisions
Sarah Böhm (1), Helena E. A. Aho-Özhan (1), Jürgen Keller (1), Johannes Dorst (1), Ingo Uttner (1), Albert C. Ludolph (1), Dorothée Lulé (1) (1) Department of Neurology, Ulm University, Ulm, Germany

P.68. Assessment of cognitive functions in ALS patients using a new eye movement based ECAS version
Keller J. (1), Krimly A. (1), Reimer L. (1), Aho-Özhan HEA. (1), Böhm S. (1), Gorges M. (1), Kassubek J. (1), Pinkhardt EH (1), Uttner I. (1), Ludolph AC. (1), Lulé D. (1) (1) Department of Neurology, Ulm University, Ulm, Germany

Genes and Genomics II (P70-P84)

P.70. Whole-Blood Global DNA Methylation in ALS and Trinucleotide Repeat Disorders
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P.72. Transcriptome of motor neurons axons derived from pluripotent cells and human spinal motor neurons
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P.74. Role of multiple mutations in disease-causing genes in Italian ALS patients
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P.76. NEK1 mutations in familial ALS
David Brenner 1, Kathrin Müller 1, Thomas Wieland 2, Patrick Weydt 1, Sarah Böhm 1, Dorothee Lulé 1, Annemarie Habers 1, Christoph Neuwirth 3, Markus Weber 3, Guntram Borck 4, Magnus Wahlqvist 5, Karin M. Danzer 1, Alexander E. Volk 4,6, Thomas Meitinger 7,8, Tim M. Strom 7,8, Markus Otto 1, Jan Kassubek 1, Albert C. Ludolph 1, Peter M. Andersen 1,9, and Jochen H. Weishaupt 1 1 Neurology Department, Ulm University, Ulm, Germany 2 Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany 3 Kantonsspital St. Gallen, Muskelzentrum / ALS Clinic, St. Gallen, Switzerland 4 Institute of Human Genetics, Ulm University, Ulm, Germany 5 Department of Neurology, Malmö University Hospital, Malmö Sweden 6 Institute of Human Genetics, Medical Centre Hamburg-Eppendorf, Hamburg, Germany 7 SyNergy, Munich Cluster for Systems Neurology, Ludwig Maximilians Università¤t München, Germany 8 Institute of Human Genetics, Technische Università¤t München, München, Germany 9 Department of Pharmacology and Clinical Neurosience, Umea University, Umea, Sweden

P.78. PLCM-seq for robust and efficient transcriptomic profiling of mouse and human motor neurons
Susanne Nichterwitz (1), Geng Chen (2), Julio Aguila Benitez (1), Marlene Yilmaz (2), Helena Storvall (2,3), Ming Cao (1), Rickard Sandberg (2,3), Qiaolin Deng^ (2) and Eva Hedlund^ (1) (1) Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden. (2) Department of Cell and Molecular Biology, Karolinska Institutet, 171 77 Stockholm, Sweden. (3) Ludwig Institute for Cancer Research, Box 240, 171 77 Stockholm, Sweden.

P.80. FUS regulates splicing of minor introns: Implications for ALS
Stefan Reber[1,2], Jolanda Stettler[1], Giuseppe Filosa[3,5], Martino Colombo[1,2], Daniel Jutzi[1], Silvia C. Lenzken[3], Christoph Schweingruber[1,2], Romy Bruggmann[4], Angela Bachi[5], Silvia M.L. Barabino[3], Oliver Mühlemann[1], Marc-David Ruepp[1] 1 Department of Chemistry and Biochemistry, University of Bern, CH-3012 Bern, Switzerland 2 Graduate School for Cellular and Biomedical Sciences, University of Bern, CH-3012 Bern, Switzerland 3 Department of Biotechnology and Biosciences, University of Milano-Bicocca, I-20126 Milan, Italy 4 Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, CH-3012 Bern, Switzerland 5 IFOM-FIRC Institute of Molecular Oncology, I-20139 Milan, Italy

P.82. ALS leads to loss of the TDP-43 repressive function on nonconserved cryptic exons in CNS locations
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P.84. A pedigree discordant for p.A4V SOD1 mutation and a novel p.E46D OPTN missense mutation
U. Manera, A. Calvo, C. Moglia, S. Cammarosano, A. Ilardi, D. Bertuzzo, M. Grassano, G. Restagno, M. Brunetti, M. Barberis, A. Canosa, A. Chiò ALS Center, Rita Levi Montalcini Department of Neuroscience, University of Turin, Turin, Italy (Chiò, Calvo, Bertuzzo, Cammarosano, Canosa, Ilardi, Grassano, Manera, Moglia); Azienda Ospedaliero Universitaria Citta della Salute e della Scienza, Turin, Italy (Chiò, Calvo); Neuroscience Institute of Torino (NIT), Turin, Italy (Chiò); Molecular Genetics Unit, Department of Clinical Pathology, Azienda Ospedaliera Ospedale Infantile Regina Margherita Sant Anna, Turin, Italy (Restagno, Brunetti, Barberis)

Mechanisms of Disease II (P86-P110)

P.86. Understanding the mechanism of SOD1 misfolding - implication to ALS pathogenesis
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P.88. The HSPB8-BAG3-HSP70 complex maintains stress granule integrity and dynamism
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P.90. TDP43 inclusions are re-routes to autophagy by the activity of the small chaperone HspB8
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P.92. TDP-43 post-translational modifications: what role for SUMOylation?
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P.94. Synthetic peptides prevent the mitochondrial dysfunction in Amyotrophic Lateral Sclerosis
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P.96. Novel cellular models of FUSopathy provide insights into regulation of paraspeckles in ALS
Tatyana Shelkovnikova, Haiyan An, Vladimir Buchman, Cardiff University, UK

P.98. Modeling FUS-ALS hallmark neuropathology using patient-specific iPSCs and iPSC-derived cortical neurons
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P.100. Intranuclear (GGGGCC)n RNA foci induce formation of paraspeckle-like structures
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P.102. Deciphering the pathological response of the astrocytes in Amyotrophic Lateral Sclerosis
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P.104. Characterization of physiological and pathological functions of HECW1 in ALS
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P.106. Cellular stress impairs the physiological function of TDP-43
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P.108. C9ORF72 rescues the loss of function of the autophagy initiator p62/SQSTM1
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P.110. ALS-causing missense mutations of CHCHD10 affect protein structure and stability
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Therapy II (P112-P134)

P.112. Safety and efficacy of botulinum toxin A for spasticity in amyotrophic lateral sclerosis
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P.114. Role of the mitochondrial Na/Ca/Li-exchanger (NCLX) in the pathophysiology of ALS
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P.116. Value of sequential designs for amyotrophic lateral sclerosis clinical trials
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P.118. Reducing mGlu5 receptors improves survival, symptoms and biological features in SOD1G93A mice
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P.120. Neuroregeneration by targeting the TGF-β system?
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P.122. MHCI deficiency accelerates muscle denervation in mouse models of amyotrophic lateral sclerosis
Maria Chiara Trolese (a)*, Giovanni Nardo (a), Mattia Verderio (a), Julio Aguila Benitez (b), Jik Nijssen (b), Laura Comley (b), Paul Heath (c), Staffan Cullheim (b), Eva Hedlund (b), Pamela J. Shaw (c), Caterina Bendotti (a). (a)_ Laboratory of Molecular Neurobiology, Department of Neuroscience, IRCCS - "Mario Negri" Institute for Pharmacological Research, Milan, Italy; (b)_ Department of Neuroscience Karolinska Institutet, Stockholm, Sweden; (c)_ Sheffield Institute for Translational Neuroscience, Department of Neuroscience, Academic Neurology Unit, Faculty of Medicine, Dentistry and Health University of Sheffield, Sheffield, UK

P.124. Interleukin-6 blockade improves inflammatory but not metabolic condition in ALS
Patin Franck 1,2, Baranek Thomas 3, Vourcâh Patrick 1,2,4, Nadal-Desbarat Lydie 3,4, Goossens Jean-Francois 5, Marouillat Sylviane 1, Dessein Anne-Frédérique 6, Madji Hounoum Blandine 1, Bruno Clément 1,2, Watier Hervé 7, Si-Tahar Mustafa 3, Leman Samuel 8, Lecron Jean-Claude 9, Gouilleux Fabrice 10, Andres 1,2, Corcia Philippe 1,11, Blasco Helen e1,2 1. UMR INSERM U930, Equipe Â Neurogénétique et Neurométabolomique, Università Francois Rabelais, Tours Cedex 1 - France 2. Laboratoire de Biochimie et de Biologie Moleculaire, Hopital Bretonneau, Tours Cedex 1, France 3. INSERM, Centre d'Etude des Pathologies Respiratoires (CEPR), UMR 1100, Universitè Francois Rabelais de Tours, Tours, France 4. PPF "Analyses des Systemes Biologiques", Universitè Francois Rabelais, Tours, France 5. Centre Universitaire de Mesure et d'Analyse (CUMA), Università de Lille, France 6. Biochemistry and Molecular Biology, Hormonology-Metabolism-Nutrition & Oncology (HMNO), Center of Biology & Pathology (CBP) Pierre-Marie Degand, Lille, France 7. UMR 7292, Universitè Francois-Rabelais de Tours, France 8. UMR INSERM U930, Equipe Troubles affectifs, Universitè Francois Rabelais, Tours Cedex 1 - France 9. Laboratoire Infammation, Tissus Epitheliaux et Cytokines, UPRES EA4331, Pole Biologie Santa, Università de Poitiers, Poitiers, France and CHU de Poitiers, Poitiers, France 10. UMR CNRS 7292, Equipe Niche Leucemique et Metabolisme Oxydatif, Tours Cedex 1, France 11. CHRU de Tours, Federation des CRCSLA Tours-Limoges, Litorals, Tours, France

P.126. Identification and characterization of specific nanobodies against the EphA4 receptor
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P.128. Extra virgin olive oil intake ameliorates reticulum stress and muscle damage in SOD1G93A mice
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P.130. Dysregulation of ROCK&ERK; in ALS: Combinatorial ROCK-ERK treatment as possible therapeutic approach
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P.132. Antisense oligonucleotides approach for the development of Amyotrophic Lateral Sclerosis therapy

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P.134. A new AAV-TDP43M337V-based simulation of Amyotrophic Lateral Sclerosis in rats

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Oral Presentations

OS.1. Project MinE GWAS: Novel risk variants and the genetic architecture of ALS

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To elucidate the genetic architecture of amyotrophic lateral sclerosis (ALS) and find associated loci, we assembled a custom imputation reference panel from whole

genome-sequenced ALS patients and matched controls (N = 1,861). Through imputation and mixed-model association analysis in 12,577 cases and 23,475 controls, combined with 2,579 cases and 2,767 controls in an independent replication cohort, we fine mapped a novel locus on chromosome 21 and identified C21orf2 as an ALS risk gene. In addition, we identified MOBP and SCFD1 as novel associated risk loci. We established evidence for ALS being a complex genetic trait with a polygenic architecture. Furthermore, we estimated the SNP-based heritability at 8.5%, with a distinct and important role for low frequency (1-10%) variants. This study motivates the interrogation of larger sample sizes with full genome coverage to identify rare causal variants that underpin ALS risk.

OS.2. What do we really know about TBK1 mutations in neurodegenerative diseases?

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Recently, loss-of-function mutations in TBK1 have been shown to be causative for ALS and FTD. Subsequent screening of additional patient cohorts led to the identification of numerous TBK1 variants associated with these diseases. 77 different TBK1 variants were identified in 103 patients suffering from ALS/FTD in a total of 5528 patients analyzed in initial screening cohorts. Pathogenicity of six TBK1 loss-of-function variants is strongly supported by co-segregation with the disease in the affected families. 27 of the 32 TBK1 loss-of-function variants generate a premature termination codon (PTC) likely resulting in degradation of the mutant mRNA by nonsense mediated mRNA decay. 50% decreased TBK1 mRNA and/or protein levels could already be confirmed with regard to seven PTC causing mutations in patient-derived cell-lines and/or post-mortem samples, indicating haploinsufficiency as the molecular genetic mechanism. However, very little is known about pathogenicity of in-frame deletion/insertion or missense TBK1 variants. For example, the 45 missense mutations identified so far do not cluster in a specific domain of TBK1, and in most instances solid statements about the relevance of specific TBK1 missense variants found in ALS patients is currently impossible. In this work we critically review what we know and what we do not know about pathogenicity of TBK1 mutations.

OS.3. An optineurin insufficiency model of amyotrophic lateral sclerosis

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Mutations in an ubiquitin-binding adaptor protein optineurin were recently found in

amyotrophic lateral sclerosis (ALS). Unlike many proteins known to cause ALS by their toxic and/or prion-like properties, optineurin is largely thought to cause disease by loss-of-function, arguing for its protective role. Although in vitro studies proposed that optineurin regulates a variety of cellular processes including cell signaling, autophagy, vesicle trafficking and maintenance of the Golgi apparatus, its direct link to ALS pathogenesis is unclear. Here we show the initial characterization of mouse models of optineurin insufficiency (Optnà157 and Optn470T), in which the TANK binding kinase (TBK) 1 or ubiquitin-binding region are lacking, respectively. Macrophages and microglia from the mice lacking the ubiquitin-binding domain of optineurin (Optn470T) had diminished TBK1 activation and IFN-beta secretion upon Toll-like receptor (TLR) stimulation. The latter was also found in the Optnà157 model, suggesting that indeed the ubiquitin-binding mutation acts by loss of function rather than as a dominant negative protein. Perhaps surprisingly, autophagy in macrophages upon TLR4 stimulation and proteosomal inhibition was not perturbed in neither of these two models of optineurin insufficiency. Importantly, Optn470T mice showed defects in motor coordination late in life (month 13-14). Further analysis of the role of TBK1 pathway and the putative anti-inflammatory role of IFN-beta in aged optineurin Optn470T mice will address if optineurin has a neuroprotective role by suppressing neuroinflammation.

OS.4. Identification of novel genetic risk factors in sporadic ALS, a discordant monozygotic twin approach

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Next-generation sequencing of ALS pedigrees and Genome-wide association studies (GWAS) have shown to be successful tools in identifying genetic risk factors in ALS. However, especially in the case of sporadic ALS, these known factors only partially explain the genetic contribution as estimated by ALS concordant and discordant twin studies. This discrepancy could possibly be attributed to rare genetic variants that might be caused by de novo mutations. We set out to identify novel (epi)genetic risk factors for sporadic ALS by analyzing the complete genomes and methylation patterns of 21 monozygotic twin pairs discordant for ALS. So far, we have investigated the single nucleotide variants (SNVs) using whole genome sequencing. To increase the chance of detecting de novo SNVs, we used three different variant calling methods and a custom filtering pipeline to obtain the most reliable discordant variants. In total we identified 149,388 SNVs of which 129,387 were eligible for further validation on a custom Axiom Genotyping Array. Preliminary results of this first step validation show on average 1.5 de novo SNVs per individual. This post-zygotic mutation rate however, was determined after exclusion of three outliers. One non-ALS twin has over 600 possible de novo mutations, which might be the consequence of comorbidity, namely leukemia. Notably, the other two outliers were both ALS affected twins, with 44 and 75 possible de novo SNVs. Further validation and analysis is required to confirm these de novo mutations and determine their possible significance as a genetic risk factor for ALS. Interestingly, our search for monozygotic twins with ALS has led to a second dataset where twins both have a C9ORF72 hexanucleotide expansion: one with concordance of the ALS phenotype, one where the sibling has FTD and one where the sibling has no signs of ALS/FTD. This data adds to the complexity of C9ORF72 pathogenicity.

OS.5. Latent Cluster Analysis of ALS Phenotypes and GWAS:Identification of Prognostically Differing Groups

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Amyotrophic lateral sclerosis (ALS) manifests as several different phenotypes. We used latent class cluster analysis to identify subgroups of ALS based on phenotype,

and tested for SNP association with subgroups at genome wide level. Phenotypic data (age of onset of weakness, sex, ethnicity, family history of ALS, site of onset of first symptoms, diagnostic delay, physician-classified phenotypic group) were obtained from UK (1313 cases and 5431 controls), Dutch (1546 cases and 2374 controls), Belgian (388 cases and 242 controls) and Irish (468 cases and 774 controls) populations, and used to perform latent cluster analysis as previously described (Ganesalingam et al. 2009). The model was tested for clinical relevance by survival analysis of the phenotypic groupings using the Kaplan-Meier method. Quality control was performed separately on genotyped data of each population and a genome wide association joint analysis was carried out in the four datasets. After quality control, there were 3,480 cases and 8,533 controls, and 264,528 SNPs. Logistic regression was used to test association with the disease and principal components were used to correct for population stratification. Genotyped data were extracted for each resulting cluster and logistic regression was performed separately. Principal components were calculated separately for each cluster. Latent class cluster analysis showed a five-cluster model had the best fit. The five clusters had significantly differing, non-overlapping survival durations. In the joint analysis, no genome-wide association was seen. In the subgroup analysis, cluster 1 showed association passing genome-wide significance for a SNP in the C9orf72 gene, (rs3849942; OR= 1.26, p=3.65 e-08), and cluster 2, in SNPs in the KCND3 gene (rs12408551, OR = 3.76, p=1.81 e-08; rs11102355, OR=3.75, p=3.04 e-08). Latent cluster analysis is an effective method for grouping patients into subgroups that convey biological or clinical significance.

OS.6. Hidden Treasures: hunting for cryptic splicing resulting from the knockdown of TDP-43

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The RNA-binding protein TDP-43 is involved in the splicing and stabilisation of messenger RNA. Rare mutations in TDP-43 are a cause of familial amyotrophic lateral sclerosis (ALS). Although TDP-43 is predominantly localised to the nucleus, in the majority of sporadic ALS cases nuclear depletion of TDP-43 is observed, along with the formation of TDP-43-containing cytoplasmic inclusions in the motor cortex and spinal cord. These observations suggest that a nuclear loss of TDP-43 function may play an important role in ALS. However, the mechanism in which this leads to neurodegeneration is unknown. A recent publication (Ling et al, 2015) showed that deletion of TDP-43 in mouse and human cells leads to the recognition and increased inclusion of normally absent or lowly expressed exons, referred to as "cryptic exons", in a small number of genes. However, further characterisation of these splicing events is required in order to understand their prevalence and importance. To confirm and expand upon these initial findings, we developed a bioinformatic pipeline to systematically identify and classify novel splicing in several publicly available RNA sequencing datasets. We present a consensus list of cryptic splicing changes in mouse

and human cell lines in response to TDP-43 knockdown or deletion. Sequence motif analysis showed that these sites are enriched in canonical TDP-43 binding sites. This was supported by iCLIP data which revealed that the majority of the cryptic sites are indeed directly bound by TDP-43. This suggests that under normal conditions TDP-43 binds to cryptic splice sites and inhibits their recognition and inclusion.

OS.7. Genetic modifier screens link nuclear transport defects to DPR pathology in C9orf72 ALS/FTD

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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). The exact role of theses aggregates in neurodegeneration, and which mechanisms underlie this specific pathology both remain unresolved. Identifying upstream pathways of these pathological events could point at novel therapeutic targets. Hexanucleotide repeat expansions in C9orf72 are the most common genetic cause of the disease, and patients present with the hallmark TDP-43 pathology observed in most ALS and a large share of FTLD cases. Unconventional translation of these repeats yields five potentially toxic dipeptide repeat proteins (DPRs). We performed two

genome-wide yeast screens and a targeted genetic screen in Drosophila, and discovered a critical role for the nuclear transport system in DPR toxicity (Boeynaems et al., Sci Rep. 2016; JoviÄ iÄ‡ et al., Nat Neurosci. 2015). These data suggest that DPRs could perturb the nucleocytoplasmic transport system, eventually resulting in cytoplasmic RBP mislocalization and aggregation. In recent work we found a strong overlap in the genetic modifiers of our previously reported PR screen and a new screen in GR flies. We identified several key transport factors which are strong enhancers of both PR and GR phenotypes. Bioinformatic analyses of the cargo sets of these transport factors, illustrates that problems in nucleocytoplasmic transport will affect processes implicated in the disease, especially centering on RNA metabolism. Moreover, these cargo sets show a striking enrichment for proteins already implicated in ALS/FTLD, suggesting that defects in nuclear transport could initiate pathogenic cascades. This implies that a defective nucleocytoplasmic transport is a prime suspect as an initiating event in ALS/FTLD pathology.

OS.8. Comparative interactomics analysis of several ALS-associated proteins identifies converging molecular pathways

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Our knowledge of the genetic etiology of ALS has increased significantly over the past years. However, our understanding of the molecular and cellular mechanisms underlying this disease is still incomplete. To address these questions, we combined protein pull down and mass spectrometry approaches to identify binding partners of wild-type and ALS-associated mutant versions of ATXN2, C9orf72, FUS, OPTN, TDP-

43 and UBQLN2 in neuronal cells. Proteomics analyses identified many putative novel interacting proteins, in addition to a small number of known interacting proteins. A striking overlap in binding partners was observed for the interactomes of ATXN2, FUS, and TDP-43, and OPTN and UBQLN2. Many binding partners of ATXN2, FUS and TDP-43 had roles in RNA metabolism, while interacting proteins of OPTN and UBQLN2 are predominantly involved in protein degradation and protein transport. C9orf72-interacting proteins were clearly distinct and function in mitochondrial biology. To confirm that this overlap is important for ALS pathogenesis, we performed a more detailed analyses of one of the common interactors of ATXN2, FUS and TDP-43: fragile X mental retardation protein (FMRP), a translational repressor that controls synaptic function. By using both in in vitro and in vivo model systems for FUS ALS we were able to show that FMRP localizes to mutant FUS-containing aggregates in spinal motor neurons and bound endogenous FUS in a direct and RNA-sensitive manner. Furthermore, experiments in zebrafish embryo's revealed that overexpression of FMRP rescues defects in neuromuscular junction (NMJ) morphology and aberrant motor behavior caused by mutant FUS in zebrafish. In all, this study links FMRP to motor neuron dysfunction caused by FUS mutations and shows that comparative interactomics analyses can aid in the identification and characterization of disease-relevant proteins.

OS.9. Development and external validation of a model for prediction of survival in individual ALS patients

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Background: Survival of patients with amyotrophic lateral sclerosis (ALS) is highly variable and prediction of survival of individual ALS patients is currently largely unknown. This hampers individual risk-assessment, stratification of patients for trials, and timing of clinical interventions. Objective: To predict survival of individual ALS patients at time of diagnosis using clinical, cognitive, genetic and imaging data. Methods: We performed an individual participant data meta-analysis of 11,471 ALS patients with a total follow-up time of 40,301 years, originating from 14 different research groups in 9 different countries in Europe. We subsequently developed and externally validated a multivariable prognostic model using internal-external cross-validation (IECV). Our model is based on predictors that were identified from previous studies and confirmed through variable selection techniques. We used state-of-the-art methods for modeling of predictor variables and implementation of an appropriate baseline hazard function. Finally, we assess the discrimination and calibration for all developed models using IECV. Results: 8 variables entered the prediction model: site of onset (hazard ratio (HR) 1.24, p<0.001), age at onset (HR 1.03, p<0.001), definite ALS according to El Escorial criteria (HR 1.30, p<0.001), diagnostic delay (HR 0.97, p<0.001), forced vital capacity (HR 0.99, p<0.001), ALSFRS slope (HR 1.22, p<0.001), presence of frontotemporal dementia (HR 1.08, p=0.385), presence of a C9orf72 repeat expansion (HR 1.26, p<0.001). Our proposed model achieved good external predictive accuracy with a C-statistic ranging between 0.74-0.83 and a median calibration slope of 1.00 (when implemented in new patients), indicating good agreement between predicted and observed survival probability 3 years after onset. Discussion and conclusion: This study proposes a model to reliably predict survival of individual ALS patients. This model can be used via a freely available and easy-to-use online tool. Results of this study bring individualized patient management and counseling, and future individualized trial design a step closer.

OS.10. Euro-MOTOR: a case-control study of fitness measures as risk factors for ALS

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Introduction: Previously, numerous studies have reported possible associations between fitness and ALS, such as physical activity (PA). Discrepancies in findings might be explained by differences in study methods, including confounding by fitness related factors such as smoking and BMI. The objective of this international, population-based case-control study was to study the association between fitness and ALS, by integrating these fitness related factors, and to explore population differences. Methods: ALS cases and matched controls were recruited in Ireland, Italy and the Netherlands. Information on PA, smoking status and BMI (including premorbid BMI) was collected using questionnaires. PA in sports, hobbies and occupations were calculated using Metabolic Equivalent of Task (MET) scores, duration and hours/week. Statistical analysis included linear mixed models used to study longitudinal data, and (conditional) logistic regression to calculate ORs. Results: 1557 ALS patients and 2922 controls were included. Controls had a higher BMI (26.2 kg/m2) compared to patients (24.5 kg/m2). BMI of patients decreased significantly over time, beginning years before study entry and diagnosis (p<0.0001). Smoking was more prevalent in patients than controls (13.4% vs. 11.1%, p=0.02, adjusted for gender and education); this difference was also present three years pre-enrollment (p<0.0001). Total MET scores were comparable between patients and controls (p=0.19). However, the percentage of patients was higher in the upper MET quartile (28.0% vs. 25.1%, p=0.04). After adjustment for gender, education, BMI and smoking, persons who are more physically active had a higher odds of developing ALS (OR2nd=1.26; OR3rd=1.26; OR4th=1.28, all p<0.03). Discussion: Smoking and PA are associated with an increased risk for ALS. The premorbid decrease of BMI over time suggests a long prodromal phase.

OS.11. Quality control of Motor Unit Number Index in 6 muscles in a single-subject Round-Robin setup

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BACKGROUND: Reliability of biomarker measurements is paramount to their successful implementation into clinical trials. Motor Unit Number Index (MUNIX) is a neurophysiological measure that provides an index of the number of lower motor neurons supplying a muscle. Its performance across centres in healthy subjects and patients with Amyotrophic Lateral Sclerosis (ALS) has been established, but inter-rater variability between multiple raters from different centres in one single subject, comparable to a round robin test in laboratory medicine, has not been investigated. OBJECTIVE: To assess intra- and inter-rater variability in a set of 6 muscles in a single subject among 12 examiners (6 experienced with MUNIX, 6 less experienced) and to determine variables associated with variability of measurements. METHODS: Neurologists and neurophysiologist from 12 European ALS centres applied MUNIX in six different muscles (abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibialis anterior (TA), extensor dig. brevis (EDB), abductor hallucis (AH)) twice in one single volunteer on consecutive days. All raters had attended at least one training course prior to measurements. Intra- and inter-rater variability as determined by the coefficient of variation (COV) between different raters and their levels of experience with MUNIX were compared. RESULTS: Mean intra-rater COV of MUNIX was 14.0% (±6.4) ranging from 5.8 (APB) to 30.3% (EDB). Mean inter-rater COV was 18.1 (±5.4) ranging from 8.0 (BB) to 31.7 (AH). No significant differences of variability between experienced and less experienced raters were detected. CONCLUSION: We provide evidence that quality control for neurophysiological methods can be performed with similar standards as in laboratory medicine. Intra- and inter-rater variability of MUNIX is muscle-dependent and mainly below 20%. Experienced neurophysiologists can easily adopt MUNIX and adequate teaching ensures reliable utilization of this method.

OS.12. Assessing long-term G-CSF as treatment option in ALS

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Objectives: Assessment of long-term G-CSF (Filgrastim) application in ALS patients guided by longitudinal follow up with potential biomarkers. Introduction: G-CSF is an established and safe hematopoietic growth factor that potentially compensates neuronal loss in ALS patients by neuroprotection, neurogenesis and immunomodulation. In contrast to encouraging preclinical data, clinical application was disappointing, presumably due to inadequate dosing, and short treatment schedules. Monitoring bone marrow stem cells and immune modulation involved in G-CSF treatment contributes unveiling mode of action. In addition, assessment of pyramidal tract integrity by MRI FA (Fractional Anisotropy) and active motor units by neurophysiology (i-MUNIX) serve as further biomarkers. Patients and Methods: 37 ALS patients (26m, 11f, mean age at start 52.1 yrs., mean ALS-FRS-r at start 38.5) were treated with G-CSF plus standard therapy after informed consent on a named patient basis. Application modes were individually adapted (range 6-816, mean 351 Mio. IU/month s.c.). Monthly visits with ALSFRS-r, clinical chemistry, blood smears and bone marrow mobilization parameters were performed, followed by cerebral MRI and neurophysiological measurements on a 3-monthly basis throughout long-term intervention up to over 5 yrs. Results: Safety and compliance were excellent. G-CSF was well tolerated and resulted in effective hematopoietic stem cell mobilization. ALSFRS-r-decline correlated significantly ($p<0.0001$) with i-MUNIX-decline, and with FA-decline ($p<0.0005$). During disease progression G-CSF treated patients with higher ALS-FRS-r mobilized more monocytes ($p<0.05$) and hematopoietic stem cells ($p<0.05$), and less eosinophils ($p<0.005$). Mobilization of monocytes ($p<0.05$), hematopoietic stem cells ($p<0.005$), and colony forming capacity ($p<0.05$) were significantly correlated to survival. We observed a significantly lower ALS progression rate ($p<0.0001$) and a clinically relevant prolongation of survival (23.9 months from start of treatment). Conclusion: Long-term administration of G-CSF in ALS patients is safe, well tolerated, feasible and needs validation. Acknowledgements: BMBF GO-Bio, MND Network Germany, BayStMWi, PRO-ACT-Database USA

OS.13. Novel phenotypic subgroups associated with the C9orf72 expansion and survival in European ALS cohort

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Introduction: The C9orf72 repeat expansion has been reported as a negative prognostic factor in amyotrophic lateral sclerosis (ALS) survival, although previous studies have not permitted investigation of interactions between C9orf72 repeat expansion, clinical/demographic factors and survival. Here we investigate the prognostic impact of the C9orf72 repeat expansion in European subgroups defined by gender and site of onset. Methods: C9orf72 status and demographic/clinical data from ALS patients from three prospective ALS registers (Ireland, Italy and The Netherlands), and DNA banks in the UK and Belgium were analysed. Royston-Parmar survival models were built including known prognostic factors (age, diagnostic delay and site of onset), gender and the presence of an expanded repeat in C9orf72. Models explore the effects of C9orf72 on survival by gender and site of onset. Sensitivity analyses were performed to determine if the results were robust to inclusion of a) El-Escorial category and b) delay to DNA sampling time. Results: 4925 ALS cases were included, of whom 457 (8.95%) carried the C9orf72 repeat expansion. An IPD meta-analysis of C9orf72 in the base model estimated a hazard ratio (HR) of 1.36 (1.18 - 1.57) for those carrying the expansion. Models using re-categorised variables revealed that C9orf72 was prognostic only in spinal onset males (HR 1.56 (95% CI: 1.25 - 1.96). This result remained significant after including El-Escorial or delay to DNA sampling as a confounder - however delay time itself was a highly significant predictor of survival. Discussion: C9orf72 repeat expansion is associated with poorer prognosis in spinal onset males only across multiple European cohorts. This finding was robust to sensitivity analysis and prompts new questions regarding the pathogenic mechanisms associated with C9orf72-mediated ALS. Delay to DNA testing is a significant predictor of survival which represents a possible sampling bias in the timing of DNA tests in ALS patients.

OS.14. Structural and functional brain signatures of C9orf72 in amyotrophic lateral sclerosis

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Introduction. Previous studies showed that C9orf72 amyotrophic lateral sclerosis (ALS) patients exhibit a more widespread structural brain involvement than sporadic cases. However, a similar distribution of brain alterations has been revealed in non-C9orf72 cases with cognitive deficits. This study investigated structural and functional MRI abnormalities in C9orf72 patients with motor neuron disease (MND) variants relative to disease severity-matched non-C9orf72 cases. Methods. 3D T1-weighted, diffusion tensor, and resting state functional MRI were obtained from 21 C9orf72 and 75 non-C9orf72 MND patients, and 22 healthy controls. Non-C9orf72 cases were grouped in patients with: no cognitive/behavioural deficits and same disease duration ("pure motor"); same patterns of cognitive/behavioural impairment but longer disease duration ("plus"); and "fast" non-C9orf72 patients with the same disease severity but shorter disease duration and faster rate of progression relative to other non-C9orf72 cases. Results. C9orf72 patients showed cerebellar and thalamic atrophy relative to all disease severity-matched non-C9orf72 cases. All MND cases showed a motor, frontal and temporoparietal pattern of cortical thinning and a distributed pattern of motor and extramotor white matter microstructural damage relative to healthy controls, independent of the genotype and the presence of cognitive impairment. Compared with non-C9orf72 "fast" cases, C9orf72 patients revealed a thinning of the occipital cortex. C9orf72 MND patients had an enhanced functional connectivity of the visual network relative to non-C9orf72 "pure motor" and "fast" cases, in spite of a "paradoxically" normal sensorimotor connectivity. Interpretation. Our data suggest that the main brain MRI signatures of C9orf72 in MND are represented by the structural cerebellar and thalamic involvement and the altered posterior cortical functional connectivity. The frontotemporal cortical and widespread white matter involvement are likely to be an effect of the disease evolution rather than be a specific marker of the C9orf72 genotype. Funding. Italian Ministry of Health (#RF-2010-2313220).

OS.15. Neuropathological spreading patterns in ALS analyzed by multiparametric MRI: DTI and 'resting-state'

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Introduction: Diffusion tensor imaging (DTI) and intrinsic functional connectivity magnetic resonance imaging (ifcMRI) can be used to identify ALS-associated patterns of brain alterations at the group level. Neuropathological data have shown that ALS may disseminate in a regional sequence in four disease-related patterns [1]. The present study shows the comprehensive application of a multiparametric MRI-based approach to analyze in vivo white matter microstructure and grey matter functional networks that are prone to be involved at each neuropathological pattern of ALS [2, 3]. Methods: Three-hundred-and-sixty data samples from ALS patients and 129 data

samples from controls acquired at 1.5T or 3.0T were analyzed by DTI and ifcMRI. DTI-based structural properties were identified by a tract of interest (TOI)-based fiber tracking approach. Tracts were analyzed that become involved during the course of ALS: the corticospinal tract (stage 1), the corticorubral and the corticopontine tracts (stage 2), the corticostriatal pathway (stage 3), the proximal portion of the perforant path (stage 4). The corresponding intrinsic connectivity networks (ICN) were the motor network (corresponding to stage 1), brainstem (stage 2), ventral attention (stage 3), and default mode/hippocampal network (stage 4). 'Resting-state' fMRI data from 145 ALS patients and 65 healthy controls were investigated for a complementary data analysis. Eighty follow-up scans were acquired, out of these, 55 subjects (45 ALS-patients and 10 controls) revealed at least two DTI data sets and ifc data sets that were useful for longitudinal analysis. Results: The multiparametric comprehensive analyses of white matter and grey matter involvement showed affectation patterns related to the regional sequence in the four disease-related patterns. For DTI data, beyond the previously recognized DTI-related affectation pattern, a categorization into ALS staging patterns was possible at the individual level, in agreement with the post mortem studies, i.e. data analysis at individual level allowed for a categorization into ALS stages. For ifc-data, group comparison revealed significantly increased functional connectivity in all four investigated networks correlated with physical disability. Differences between ALS patients and controls could be verified by analysis of the longitudinal data. Conclusion: This large-scale monocentric MRI analysis showed in vivo a disease spreading pattern that is related to the pathological propagation pattern in ALS. This approach might enlarge the spectrum of potential non-invasive surrogate markers as a multiparametric neuroimaging-based read-out for clinical trials in ALS. References [1] Brettschneider J, et al, Ann Neurol 2013; 74: 20-38. [2] Kassubek J, et al, Brain 2014, 137:1733-1740 [3] MÃller H-P, et al, JNNP 2016,pii: jnnp-2015-311952.

OS.16. A connectivity-based analysis of striatal pathology in ALS

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Background: Basal ganglia pathology is increasingly recognised in ALS. The heterogeneous clinical symptoms of ALS are manifestations of complex network dysfunction as opposed to isolated grey or white matter pathology. Dysfunction of frontostriatal, nigrostriatal, and corticobasal networks contribute to the unique cognitive, behavioural, pyramidal and extrapyramidal deficits observed in ALS. Recent neuroimaging studies have demonstrated significant subcortical grey matter involvement in ALS, but relatively little is known of the selective vulnerability of striatal nuclei. Objectives: The aim of this study is to comprehensively characterise striatal and sub-thalamic networks in ALS based on probabilistic cortical-striatal connectivity profiles. Methods: Eighty C9orf72 negative ALS patients, twelve patients carrying the C9orf72 hexanucleotide repeat and forty age-matched healthy controls were included in a multi-parametric neuroimaging imaging study. Subcortical structures were delineated based on high resolution 3D structural data sets, using intensity gradients and automated boundary corrections. Pathology within the structures was evaluated using connectivity-based segmentation. Seven sub-regions were evaluated according to cortical-striatal anatomical connections. The cortical

targets included limbic, executive, rostral-motor, caudal-motor, parietal, occipital and temporal cortical zones. Both diffusivity and density analyses were carried in the identified sub-nuclear regions. Results: The most significant diffusivity and density alterations were identified in thalamic and caudate foci which connect to caudal motor regions, pathological changes were also mapped to striatal nuclei connecting to rostral motor areas. Hexanucleotide repeat carriers showed considerable pathology in limbic and executive projections. The connectivity-based pathology profile of hexanucleotide repeat carriers is distinctly different from C9orf72 negative patients. Conclusions: Striatal pathology in ALS exhibits network-wise vulnerability mirroring cortical atrophy patterns. Comprehensive basal ganglia analyses demonstrate connectivity based susceptibility patterns. Our findings support the notion that interconnected brain regions show concomitant neurodegeneration and the pathophysiological observation that "What wires together dies together".

OS.17. In vivo analysis of neuro-inflammation with [11C]-PBR28: Clinical and MR spectroscopic correlations

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Background - Inflammation has been highlighted as an important mechanism in ALS pathogenesis, but reports of inflammation in vivo in humans, and the relationship with neuronal degeneration and clinical variables is limited. This study investigated glial activation in vivo using the PET radioligand [11C]-PBR28 in ALS, and the relationship between glial activation, MR spectroscopy, and clinical scores. Methods - PET ([11C]-PBR28 binding), MR spectroscopy (CSF-corrected N-acetylaspartate, NAA) and clinical data (ALSFRS, UMNB, ECAS and MoCA) were collected for 11 ALS patients and 11 controls. We investigated regional binding using both pairwise comparisons (t-tests) and the ensemble learning technique, Random Forests. Correlations were assessed between: i) [11C]-PBR28 binding and NAA concentration in the motor cortex, and ii) regional binding and cognitive scores. Results - There was increased [11C]-PBR28 binding in the left motor, pre-frontal, and temporal cortices, and right basal ganglia, thalamus and medial temporal lobe in ALS subjects. A Random Forests model using regional binding was able to correctly classify 100% of cases, with the regions identified using pairwise comparisons contributing most to the model. Increased left pre-central [11C]-PBR28 binding was associated with a lower NAA concentration (r=-0.66, p=0.026). For clinical data, it was found that higher ventromedial prefrontal cortex binding was associated with more rapid progression of ALSFRS score (r=0.62, p=0.042); higher motor cortex binding was associated with lower ECAS score (r=-0.60, p=0.049); and higher retrosplenial cortex binding was associated with lower MoCA score (r=-0.70, p=0.016). Conclusions - ALS patients had significantly increased [11C]-PBR28 binding in brain regions previously implicated in the disease, and we provide in vivo evidence of a relationship between neuronal degeneration and inflammation

in the pre-central gyrus, as well as novel clinic-radiological correlations. Random Forests are a powerful tool to analyze data with multiple dependent variables without the issue of multiple comparisons.

OS.18. Structure and small molecule interaction of pathogenic C9orf72 RNA

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Large expansions of a non-coding GGGGCC-repeat in the first intron of the C9orf72 gene are a common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Pathological repeat expansion is associated with the formation of nuclear RNA foci and aberrant repeat-associated non-ATG (RAN) translation of dipeptide repeats. G-rich sequences have a strong propensity to form stable secondary structures known as G-quadruplexes, which have been shown to affect several different aspects of gene expression. We have shown that the C9orf72 (GGGGCC)4 repeat can form a very stable RNA G-quadruplex. Using an established FRET assay, we screened a highly curated library of small molecules developed at the UCL School of Pharmacy, for their G-quadruplexes binding properties. 8.3% of the compounds bound selectively to RNA, among which we focused on three structurally related small molecules. We have further characterized these small molecules in cortical neurons derived from three independent pluripotent stem cell lines from patients affected by Amyotrophic Lateral Sclerosis, carrying expanded repeats. All three compounds significantly decreased RNA foci.. These data suggest specific targeting of RNA G-quadruplexes may have therapeutic potential for C9orf72 FTD and ALS.

OS.19. CRISPR/Cas9 genome editing results in correction of ALS phenotypes in C9orf72 mutant iPSC-derived motor neurons

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A hexanucleotide expansion mutation in the C9orf72 gene is the single commonest cause of amyotrophic lateral sclerosis and frontotemporal dementia. In order to overcome the inherent variability in iPSC-derived neuronal lines we used CRISPR/Cas9 genome editing with homologous recombination using a donor template containing a puromycin selection cassette to create isogenic lines in which the mutant expansion

is replaced by a normal sized hexanucleotide repeat. We screened our puromycin resistant clones by repeat-primed PCR to check for the absence of abnormal repeat expansions and we found seven clones with normal repeat size and accurate homologous recombination. We used the edited clones for further evaluation of the disease phenotypes observed in iPSC-derived motor neorons from ALS patients carrying the hexanucleotide repeat expansion mutation. Gene correction resulted in complete abolition of sense and antisense RNA foci, restoration of normal methylation and gene expression levels at the C9orf72 locus, and reversal of sensitivity to glutamate toxicity, activation of markers of apoptosis and stress granule formation. This model establishes that removal of the hexanucleotide expansion is sufficient to restore normal cellular homeostasis and provides an important tool for the further exploration of ALS pathogenesis.

OS.20. Oculomotor restricted protein SYT13 protects motor neuron from selective death in ALS and SMA

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Mechanisms responsible for motor neuron subtype-selective degeneration in Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA), two fatal still incurable neurological diseases, remain unknown. Indeed, the molecular signatures of the oculomotor neurons that are resistant to degeneration is distinct from that of spinal cord/brain stem motor neurons, offering some clues to their differential vulnerability. We identified Synaptotagmin 13 (SYT13), as being highly expressed in disease-resistant human and rodent oculomotor neurons. In human in vitro models of ALS/SMA, we demonstrated rescue in motor neurons survival, axonal length and neuromuscular junctions in response to overexpression of SYT13. Adeno-associated virus9-mediated delivery of Syt13 to ALS/SMA in vivo models improved pathology, delayed muscle denervation and prolonged survival. Mechanistically, increase in SYT13 levels was associated with reduction of apoptotic signs and stabilization of neuromuscular junctions. These findings sustain the role of SYT13 as a candidate therapeutic target for motor neuron diseases. Identification of the mechanisms of neuronal differential vulnerability may lead to therapies preventing the progressive loss of motor neurons in ALS and SMA.

OS.21. Detailed analysis of misfolded SOD1 species in patient-derived cell types

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Mutations leading to structural instability and unfolding/misfolding of superoxide dismutase 1 (SOD1) are a common cause of ALS. Accumulation of soluble misfolded SOD1 promotes aggregation and the formation of prion-like species that are able to propagate through the motor system. SOD1 aggregation correlates with motor neuron degeneration. However, neither the events leading to misfolded SOD1 accumulation and aggregation, nor the selective vulnerability of motor neurons are well understood. This has typically been addressed by overexpression of human SOD1 in vivo and in vitro. Patient-derived cell models, where SOD1 is expressed at physiological levels, offer a promising in vitro alternative. Here we show that both soluble and aggregated misfolded SOD1 are present in patient-derived cell types (fibroblasts, induced pluripotent stem cells (iPS) and iPS-derived motor neurons (iPS-MNs) and iPS -astrocytes) but in differing amounts. We found the highest levels of soluble misfolded SOD1 in iPS-MN and astrocytes where it exists in the same disordered, monomeric form found in the spinal cord of human SOD1 transgenic mice. Very low levels of misfolded SOD1 were detected in non-disease, or isogenic controls, and in non-SOD1, and sporadic ALS lines. Detergent-insoluble SOD1 aggregates were found to be low, but increased following inhibition of proteasomal degradation, particularly SOD1 mutant astrocytes. These results indicate that although the template for SOD1 aggregation is enriched in human iPS-MNs and astrocytes, extensive aggregation is not an intrinsic phenomenon in these cells, at least at a relatively immature stage in vitro. Our results support the use of iPS-derived cell types to study the mechanisms of SOD1 misfolding in vitro at physiological levels of SOD1 expression. Development of more sophisticated models could help clarify the pathways of SOD1 aggregation leading to prion formation.

OS.22. Targeting extracellular cyclophilin A extends survival in the SOD1G93A mouse model of ALS

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Neuroinflammation is a major hallmark of ALS. Several anti-inflammatory compounds have been evaluated in patients and animal models, but have been proved disappointing, probably because effective targets have not yet been identified. Cyclophilin A (PPIA) as a foldase is beneficial intracellularly, but extracellularly has detrimental functions. We found that extracellular PPIA is an unexpected mediator of neuroinflammation in ALS. It is a major inducer of matrix metalloproteinase-9 and is selectively toxic for motor neurons. High levels of extracellular PPIA were found in cerebrospinal fluid of SOD1G93A mice and sporadic ALS patients, indicating that our findings may be relevant for familial and sporadic cases. A specific inhibitor of extracellular PPIA, given at symptom onset, rescued motor neurons and extended survival in the SOD1G93A mouse model of ALS. The treatment reduced glial activation, pro-inflammatory markers, NF-kB activation, endoplasmic reticulum stress and aggregation. This evidence indicates that extracellular PPIA is a promising druggable target for ALS and support further studies to develop a therapy to arrest or slow the progression of the disease in patients.

OS.23. CSF1R blockade slows the progression of amyotrophic lateral sclerosis by reducing microgliosis and invasions of macrophages into peripheral nerves

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Inflammation is a common neuropathological feature in several neurological disorders, including amyotrophic lateral sclerosis (ALS). We have studied the contribution of CSF1R signalling to inflammation in ALS, as a pathway previously reported to control the expansion and activation of microglial cells. We found that microglial cell proliferation in the spinal cord of SOD1G93A transgenic mice correlates with the expression of CSF1R and its ligand CSF1. Administration of GW2580, a selective CSF1R inhibitor, reduced microglial cell proliferation in SOD1G93A mice, indicating the importance of CSF1-CSF1R signalling in microgliosis in ALS. Moreover, GW2580 treatment slowed disease progression, attenuated motoneuron cell death and extended survival of SOD1G93A mice. Electrophysiological assessment revealed

that GW2580 treatment protected skeletal muscle from denervation prior to its effects on microglial cells. We found that macrophages invaded the peripheral nerve of ALS mice before CSF1R-induced microgliosis occurred. Interestingly, treatment with GW2580 attenuated the influx of macrophages into the nerve, which was partly caused by the monocytopenia induced by CSF1R inhibition. Overall, our findings provide evidence that CSF1R signalling regulates inflammation in the central and peripheral nervous system in ALS, supporting therapeutic targeting of CSF1R in this disease.

OS.24. Dimethyl fumarate (Tecfidera) delays onset and improves motor function in SOD1G93A transgenic mouse

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Dimethyl fumarate (DMF, Tecfidera, Biogen) has recently gained approval for the treatment of multiple sclerosis (MS) and has multiple modes of action, as an agonist of Nrf2-directed transcription, as well as an inhibitor of NfkappaB. Both activities could be beneficial in ALS. In vivo DMF is rapidly metabolised to mono-methyl fumarate (MMF) which is CNS penetrant. We have a long standing interest in Nrf2 activators in particular and set out to determine the effects of DMF in the SOD1G93A transgenic mouse model of ALS dosed at 200mg/kg once daily as an oral suspension in methylcellulose (10ml/kg, 20mg/ml). Two independent experiments indicated that DMF slowed the decline in motor function in this model. The first examined short term effects (up to postnatal day 60) on fastrac running wheel performance for a number of Nrf2 activators and indicated that DMF could slow the decline in running performance as determined by Two Way ANOVA ($P<0.0001$ versus vehicle dosed mice). The second experiment was a comprehensive study looking at rotarod performance, onset and survival. DMF was able to slow the decline in rotarod performance ($P<0.001$, two-way ANOVA) and increased the time to reach a 20% decline in rotarod function by 7 days ($p=0.0372$, Student's T Test). Onset was delayed by two weeks ($P=0.0007$, Student's T test). No effect was seen on survival. The results were similar to historical data obtained for riluzole in this model. Given the mode of action is distinct to riluzole this molecule may warrant clinical testing. However, further work is needed to understand how the exposures observed in these experiments relate to that seen in humans dosed with approved clinical doses for multiple sclerosis.

OS.25. Beneficial effects of RNS60 in cellular and animal models of amyotrophic lateral sclerosis

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Neuroinflammation mediated by innate and adaptive immune responses plays an important role in the pathogenesis and progression of ALS. Activated microglia, infiltrating T cells and perturbed cytokine/chemokine homeostasis are prominent hallmarks of the disease. RNS60 is a novel therapeutic compound with anti-inflammatory properties, produced by Revalesio Corporation and composed by 0.9% saline solution containing charge-stabilized nanostructures with oxygen core generated by subjecting normal saline to Taylor-Couette-Poiseuille flow under elevated oxygen pressure. It has been demonstrated that RNS60 attenuates inflammatory responses reducing iNOS expression in activated astrocytes and microglia by the inhibition of NF-kB (Khasnavis et al. 2012). In addition, RNS60 inhibited encephalomyelitis progression in mice by enhancing Treg function and displayed significant neuroprotective effects in animal models of Parkinson and Alzheimer's diseases (Khasnavis et al. 2014). RNS60 can also enhance ATP synthesis by facilitating oxygen transport into the mitochondrial system (Choi et al.,2014). Based on this evidence, we evaluated the neuroprotective and anti-inflammatory effects of RNS60 in both in vitro and in vivo models of familial ALS. Using astrocytes/spinal neuron and microglia/spinal neuron cocultures from SOD1G93A mice, we found that RNS60 significantly protected motor neurons from death induced by the presence of the mutated SOD1 and reduced iNOS levels produced by astrocytes. When we examined the effect of RNS60 in SOD1G93A mice, we found that chronic intraperitoneal treatment with RNS60 significantly delayed the onset of motor symptoms, ameliorated the muscle weakness, and slightly increased their survival. This occurred together with a partial but significant protection of α-motor neurons and neuromuscular junction. Thus, based on these significant preclinical findings, as well as the excellent clinical safety profile, RNS60 may be a promising candidate for ALS therapy which deserves further study.

OS.26. Approaches to enhance the cell response to proteotoxicity in ALS

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ALS, like others neurodegenerative diseases (Alzheimer's, Parkinson, Huntington,

Spinal and Bulbar Muscolar Atrophy (SBMA)) is characterized by the formation of proteotoxic aggregates in affected cells. Aggregates could derive from a reduced/insufficient activity of the protein quality control (PQC) system. The PQC is responsible for the removal of misfolded proteins and comprises chaperones (like Heat Shock Proteins, HSPs) and degradative pathways (ubiquitin proteasome system (UPS) and autophagy). Either in familial or sporadic ALS forms (fALS and sALS), PQC has been found impaired. Enhancing/restoring PQC response could be protective in ALS. We analysed the role of the small heat shock protein B8 (HSPB8), a PQC component that prevents misfolded proteins accumulation in Alzheimer's, Huntington and SBMA cellular models. We found high levels of HSPB8 in transgenic ALS mice motoneurons, which survive until end-stages of disease. Using ALS cellular models, we demonstrated that HSPB8 overexpression counteracts the aggregation of mutant SOD1G93A, truncated TDP43 fragments (àC TDP43, associated to fALS, and TDP35 and TDP25, associated to sALS), as well as expanded polyGP C9Orf72 products. HSPB8 increases their autophagic degradation, by complexing with the co-chaperones BAG3, HSC70 and CHIP. The complex allows substrates recognition by p62/SQSTM1 and autophagy degradation. We confirmed HSPB8 protective function in Drosophila melanogaster (Dm) ALS models. In fact, overexpression of HSP67Bc, the functional ortholog of human HSPB8, reduces eye degeneration in different Dm TDP43 models. HSPB8 could be a good therapeutic target for ALS. We next set up a high-throughput screening to identify inducers of HSPB8 expression in neuronal cells. We identified two compounds, colchicine and doxorubicin, that up-regulate HSPB8 expression. Both compounds counteract the accumulation of TDP43 misfolded species. Safer and more tolerable analogs of colchicine and doxorubicin, still able to induce HSPB8 may result beneficial in ALS models. GRANTS: Ministero della Sanità (GR-2011-02347198); AFM; TELETHON; CARIPLO; ARISLA; JPND.

OS.27. Phenotypic profiling of compartmentalized iPSC-derived ALS neurons with live imaging tools

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The use of induced pluripotent stem cells (iPSC) has pioneered modelling of human diseases. Since valid models of neurodegeneration are still rare, iPSCs offer great opportunities especially for investigating the underlying pathogenesis. Our group utilizes iPSC-derived neurons from patients of hereditary Amyotrophic Lateral Sclerosis (ALS). We aim to understand how perturbed axonal trafficking and organelle metabolism contributes to neurodegeneration, as the lengthy architecture of these outgrowths renders them particularly vulnerable for compromised delivery of energy, mRNA and other cargo to distal outposts. Using Xona Microfluidic Channel (MFC) cultures, we perform fast multi-channel live imaging on compartmentalized neurons with standardized readout windows in distal versus proximal axon parts with defined directionality (retrograde vs anterograde). Our tracking analysis has revealed distinct phenotypes across our library of iPSC clones, depending on affected gene and mutation. For example, one type often found for ALS mutations is perturbed motility and membrane potential of mitochondria in distal axons whereas in the proximal part trafficking functions normal ('gradient phenotypes'). Conversely, another distinct phenotype features abnormal elongation of mitochondria along with a moderate decrease of membrane potential on both the proximal and distal site ('global phenotypes'). Beyond a purely descriptive cataloguing, our phenotypic profiling comprises a standardized chemical and genetic interrogation protocol with bioactive compounds of known targets, thereby revealing the underlying pathways of distinct axonopathies. Of particular interest are compounds that either mimic or rescue a phenotype. Based on the paradigm of Modular Cell Biology, the response of a newly interrogated disease model allows its assignment to a phenotypic class, thereby pointing to the underlying mechanism. Collectively, we have established a powerful, analytic imaging platform of compartmentalized neurons for comprehensive modeling of neurodegeneration, molecular dissection and identifying novel therapeutic targets.

OS.28. Evaluation of a TDP-43Q331K mouse model: is it useful for therapeutic trials in ALS?

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Background: Preclinical therapeutic trials of Amyotrophic Lateral Sclerosis (ALS) are mostly carried out in mouse models based on SOD1 mutations. Nearly all non-SOD1 patients have inclusions of TDP-43, hence models based on TDP-43 mutations may be more predictive for clinical trials in patients. Methods: Two mouse lines transgenic for human TDP-43[1] (TDP-43WT and TDP-43Q331K) were subjected to phenotypic and pathological characterisation. Findings: the TDP-43Q331K mice showed signs of tremor, muscle loss, a swimming gait, motor dysfunction and weight gain. No phenotype was observed in TDP-43WT mice. TDP-43Q331K mice showed significantly worse rotarod performance (30.5%-68.2% reduction between 12-43 weeks, $P<0.05$, two way ANOVA, n=5-7/group), and significantly increased weight (37.3%-27% increase between 12-42 weeks, $P<0.05$, n=5-7), despite triceps surae muscle loss (234.37±12.8mg in TDP-43Q331K vs 413.3±18.1mg in TDP-43WT mice at 10 months, $p<0.001$, n=6-10). Electrophysiology of gastrocnemius at 10 months of age found a 73% loss of compound muscle action potential (CMAP) amplitude in the TDP-43Q331K mice compared to the TDP-43WT mice ($p<0.001$). An ongoing study suggests TDP-43Q331K mice consume similar amounts of food, and are equally active compared to TDP-43WT mice. Quantification of huTDP-43 localisation by immunohistochemical staining indicated a 46% increase in nuclear ($p<0.01$) and a 19% increase in cytoplasmic staining ($P>0.05$) levels in spinal motor neurons of TDP-43Q331K mice compared to TDP43WT controls. This suggests nuclear clearing of TDP-43 is not a feature driving

early motor system degeneration. Power calculations suggest group sizes of 12 females are sufficient to detect effect sizes of 10-20% in the majority of parameters measured in a 6 month therapeutic trial. Conclusion: TDP-43Q331K mice show promise as a model of MND for therapeutic trials. Measurement of CMAP is a potential translatable biomarker of disease progression that could also be used in human clinical trials. 1. Arnold et al. Proc Natl Acad Sci USA 2013;110:E736-745.

OS.29. Alpha-synuclein interacts with SOD1 and promotes its oligomerization

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Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are both neurodegenerative diseases affecting the movement system. Alpha-synuclein plays a central role in the pathogenesis of PD whereas Cu, Zn superoxide dismutase (SOD1) is a key player in a subset of ALS cases. Under pathological conditions both alpha-synuclein and SOD1 form oligomers and fibrils. We investigated the possible molecular interaction of alpha-synuclein and SOD1 and its functional and pathological relevance. Using a protein-fragment complementation approach, immunhistochemistry and co-immunoprecipitation, we found that alpha-synuclein and SOD1 physically interact in living cells, human erythrocytes and mouse brain tissue. Additionally, our data show that disease related mutations in alpha-synuclein (A30P, A53T) and SOD1 (G85R, G93A) modify the binding of alpha-synuclein to SOD1. The interaction of alpha-synuclein and SOD1 does not affect the SOD1 activity. Alpha-synuclein might rather change the polymerization property of SOD1, as it increases SOD1 dimerization/ oligomerization. In conclusion, we provide evidence for direct interaction of alpha-synuclein and SOD1, suggesting interconnected pathogenic functions in neurodegenerative diseases.

OS.30. Two superoxide dismutase prion strains transmitting amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) patients and transgenic mice carrying mutant human superoxide dismutase-1 (hSOD1) develop aggregates of unknown significance. Using a novel assay for structural characterization - binary epitope mapping - we have found that two different strains of hSOD1 aggregates, denoted A and B, can arise in mice. Minute amounts of strain A and B hSOD1 aggregate seeds, prepared by centrifugation through a density cushion, were inoculated into lumbar spinal cord of 100-day-old mice carrying a hSOD1 transgene. The mice developed premature signs of ALS and became terminally ill after around 100 days - 200 days earlier than mice which had not been inoculated or were inoculated with a control preparation. Concomitantly, exponentially growing strain A and B hSOD1 aggregations, respectively, propagated rostrally throughout the spinal cord and brain stem. The structures of the A and B strains are widely different, and the disease phenotypes they caused differed regarding aggregation and symptom progression rates, aggregate distributions along the neuraxis, and histopathological pictures. Thus, the hSOD1 aggregate strains are prions and spreading templated aggregation is the core disease mechanism in SOD1-provoked ALS.

OS.31. C9ORF72 function synergizes the toxicity of Ataxin-2 with intermediate polyQ repeats

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ATXN2 has been found to be a modifier of TDP-43 toxicity, as described in yeast and in Drosophila (Elden et al. Nature 2010). Through a meta-analysis using hundreds of French ALS patients we defined the size of ATXN2 polyglutamine (polyQ) repeats as the most common risk factor in ALS (Lattante et al., Neurology 2014). Surprisingly, ATXN2 intermediate repeats (Q30x) coincided with C9ORF72 expansions. Therefore, we proposed that ATXN2 polyQ expansions could act as a pathological modifier in the presence of a C9ORF72 repeat expansion. Previously, a consequence of decreased C9ORF72 levels was observed in patients where pathological repeats lead to decreased C9ORF72 both at the transcript and protein levels (Ciura et al., Ann Neurol 2013; Xiao et al. Ann Neurol 2015). To determine whether ATXN2 intermediate repeats and C9ORF72 interact in the same pathological pathway, we tested the deleterious effect of a partial depletion of C9ORF72 in both embryonic mouse cortical neurons and in an in vivo model of zebrafish with or without the overexpression of ATXN2 Q30x and Q20x. In cellular and animal models partially reducing the expression of C9ORF72 was not able or sufficient to induce cell death, protein aggregation or motor neuron malformations. In contrast, depletion of C9ORF72 synergizes the aggregation and toxicity properties of ATXN2 Q30x, but not those associated with ATXN2 Q22 in both animal and cellular models. In zebrafish models, co-expression of C9orf72 with ATXN2 Q30x solely resulted in reduced swimming distance, velocity as well as disrupted arborization and shortening of the motor neuron axons. The molecular mechanisms through which mutant ATXN2 and C9OR72 may cause motor neuron degeneration in ALS are not well understood. Therefore the combination of in vivo and in vitro models we have developed could be a valuable tool for the pathological characterization of these pathogenic interactions.

OS.32. Direct RNA toxicity in a transient zebrafish model of C9orf72 ALS/FTD is abrogated by Puraα

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Background: Toxicity of the GGGGCC hexanucleotide repeat expansion is believed to be mediated by direct effect of mRNAs containing the repeat expansion ('RNA toxicity'), or by the formation of dipeptide repeat proteins (DPRs) through repeat-associated non-ATG (RAN) translation ('DPR toxicity'). The existence of RNA toxicity has so far not been shown in any in vivo model. Objectives: We wanted to gain in vivo evidence for direct RNA toxicity and investigate the modifying role of PurÎ± in C9orf72 ALS/FTD. Methods: RNA constructs containing different lengths of sense and antisense repeats and ATG-containing codon-optimized constructs for all DPRs were injected into one-cell stage zebrafish embryos. Motor axonal length and branching at 30 hours post fertilization were used a read-out. Dot blot using PR- and GR- specific antibodies was performed. RNA constructs which can't produce DPRs because the repeats are interrupted with stop codons ('RNA only constructs') were also tested. We generated five Puraα constructs each lacking one of the five domains of Puraα protein. Results: RNA constructs display toxicity both in the sense and antisense direction, however with a different length-dependent threshold. (GR)50 and (PR)50 constructs were toxic. GR and PR were detected on dot blot in these fish, but were not present in fish injected with repeat RNA constructs. Sense and antisense 'RNA only constructs' were also toxic. Overexpression of Puraα diminished sense, antisense and 'RNA only' toxicity, but not PR- and GR-toxicity. This effect relied on a glycine-rich domain and an RNA binding domain, both implicated in stress granule dynamics. Conclusion: This study provides in vivo evidence for the presence of RNA toxicity in C9orf72 ALS/FTD, independent of DPR toxicity. However, our data do not exclude that both forms of toxicity exist. We identified Puraα as a selective modifier of RNA toxicity, possibly through a link with stress granules.

OS.33. Depletion of C9orf72 causes immune system dysfunction in mice

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Hexanucleotide (GGGGCC) repeat expansions in the noncoding region of the C9ORF72 gene are the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD; C9ALS/FTD). Decreased C9orf72 protein expression in the frontal cortex of C9ALS/FTD patients supports the idea that C9orf72 haploinsufficiency may contribute disease pathogenesis. To examine whether C9orf72 loss-of-function leads to neuron degeneration and motor deficits, we generated neural-specific and full C9orf72 knockout mice. In both mouse lines, ablation of C9orf72 resulted in decreased body weight without affecting motor function or inducing pathological hallmarks of ALS, such as motor neuron degeneration, gliosis, TDP-43 mislocalization or enhanced ubiquitination. Unexpectedly, full C9orf72 knockout mice showed reduced lifespan and post-mortem analyses revealed that full C9orf72 knockout mice develop lymphadenopathy and splenomegaly with extramedullary hematopoiesis. Disrupted tissue architecture and severe histiocytic infiltration was present in multiple organs including spleen, lymph nodes, bone marrow, kidney and lung. Overall, these observations suggest that C9orf72 depletion causes severe dysregulation of the histiocyte-macrophage cell lineage leading to systemic neoplastic events. Our findings suggest that C9orf72 loss-of-function by itself does not cause C9ALS/FTD pathology but support an important novel role for C9orf72 in histiocytic-macrophage function. These observations have important therapeutic implications as they suggest that therapeutic strategies aimed at lowering systemic C9orf72 levels in C9ALS/FTD patients may have undesirable side effects in the immune system.

OS.34. Role of Fus in post synaptic neuromuscular junction differentiation

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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). 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. Here we hypothesized that cytoplasmic mislocalization of FUS leads to defects in neuromuscular junction (NMJ) development. Indeed, mice with 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 and decreased expression of NMJ related genes. Consistent with a key role of FUS in the NMJ, we observed accumulation of Fus in endplates of wild type mice and this accumulation was disrupted after denervation, as well as in heterozygous Fus knock-in mice. These results suggest that FUS plays a key role in the development and maintenance of the post-synaptic part of the neuromuscular junction.

OS.35. Cellular characterisation of a novel FUS mouse model of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder resulting in the loss of upper and lower motor neurons in the spinal cord, which leads to the progressive degeneration of the neuromuscular system. FUS is an RNA binding protein primarily located in the nucleus, and involved in several aspects of RNA metabolism; ranging from transcription and splicing, to RNA transport, stress granule formation and protein translation. Mutations in FUS are associated with early onset forms of ALS and are often located in the C-terminal nuclear localisation signal of the protein, resulting in its cytosolic mislocalisation. In addition, FUS-containing cytosolic protein aggregates have been detected in both ALS post-mortem tissue and mouse models of disease. Here we characterise a novel FUS mouse model of ALS, carrying a frame shift mutation found de novo in an aggressive and early onset case of ALS. This mutation occurs in the splicing acceptor site within intron 13, causing the skipping of exon 14 (FUS à14), thus resulting in the loss of FUS nuclear localisation signal and translation of an out-of-frame region at the C-terminus of the protein. FUS à14 was found to be primarily located in the cytosol of primary motor and sensory neurons, as well as glial cells. Although cytosolic, FUS à14 was shown not to form aggregates, but interestingly, it was found to be present in discrete structures throughout the cytoplasm. Since axonal transport has been shown to be affected in several models of ALS and often to precede the pathological hallmarks of the disease, we investigated the trafficking of different cargoes along motor neuron axons grown in microfluidic chambers. Our results provide new insights into the function of FUS mutants in primary motor neurons and their implications in neuronal physiology.

OS.36. Drosophila FUS mutant phenotypes are mediated by increased Xrp1 expression in neurons

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Fused in sarcoma/translocated in liposarcoma (FUS/TLS) is a DNA- and RNA-binding protein involved in regulation of transcription, mRNA splicing and mRNA subcellular localization. Under physiological conditions, FUS displays a predominantly nuclear localization. FUS-containing cytoplasmic protein aggregates in neurons and glia, often associated with loss of nuclear FUS, are a pathological hallmark in about 10% of patients with frontotemporal dementia (FTD), and in patients with familial forms of the motor neurodegenerative disorder amyotrophic lateral sclerosis (ALS) caused by mutations in FUS. Loss of function of the Drosophila FUS homolog cabeza (caz) results in inability of pharate adult flies to eclose from the pupal case due to motor deficits. Here, we performed a genetic modifier screen for rescue of caz mutant pupal lethality, which identified Xrp1 as a key modifier of caz mutant phenotypes. Heterozygosity for Xrp1 not only rescued caz mutant pupal lethality, but also adult motor performance and life span. Interestingly, selective knock-down of Xrp1 in neurons was sufficient to rescue caz mutant phenotypes. Xrp1 expression is strongly upregulated in caz mutants, and selective Xrp1 overexpression in neurons of otherwise wild type flies results in developmental lethality, with adult escaper flies displaying motor performance defects and shortened life span. The genetic interaction between caz and Xrp1 depended on the functionality of the AT-hook DNA-binding domain in Xrp1, and high-throughput RNA sequencing revealed profound gene expression dysregulation in caz mutant animals, which was substantially rescued by Xrp1 heterozygosity. Together, our findings indicate that caz mutant phenotypes are mediated by increased neuronal Xrp1 levels, leading to gene expression dysregulation and neuronal dysfunction.

Posters

Biomarkers

P.01. Whole CSF proteome analysis for the identification of specific biomarkers in ALS

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Background: The disease course of ALS is gradual but progression is. Up to one third of patients survive for either more than 48 months, or die before 18 months after symptom onset. A successful therapy is one which slow disease progression, yet it is unknown which factors protect patients from a rapid disease course. Therefore specific biomarkers are required for early diagnosis of ALS and for monitoring and evaluation of the efficacy of new treatments. Objectives: A comprehensive proteomic search strategy is applied for the search of new specific biomarkers in CSF of patients with ALS. CSF was acquired from rapidly and slowly progressive ALS patients and controls. The proteomes were analysed and compared to identify potential specific biomarker candidates discriminating these 3 groups. Proposed biomarker candidates were evaluated. Methods: Pooled CSF samples of each group were used. CSF-pools were concentrated and 2D fractionated (SEC, AEC). For each pool we got 1560 2D-fractions. Fractions with protein conc. >0.03 mg/ml were analysed by LC-MS/MS; data were processed by Proteome Discoverer and Sieve. The validation of the protein concentration of proposed candidates in individual samples was performed by ELISA. Results: We identified 1824 protein groups supported by >/=1peptide and 676 with >/=2 peptides. Control CSF vs. CSF of ALS patients showed significantly increased or decreased proteins, some of which are strongly associated with brain injury and neuronal death. ELISA evaluation in a new cohort showed statistically relevant alterations in a subgroup of the identified proteins. Conclusion: We identified and validated significantly increased or decreased proteins in CSF of ALS patients. The use for diagnostic as well as for prognostic purposes will be further examined. Acknowledgement: This research is supported by BMBF (Bundesministerium fÃ¼r Bildung and Forschung) in the framework of the E-RARE programme (PYRAMID) and JPND (SOPHIA) of the European Union.

P.02. Serum C-reactive protein (CRP) as a simple and independent prognostic biomarker in ALS

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Different factors have been proposed as possible candidates to predict prognosis in ALS, however, there is still no consensus on which biomarkers are reliable prognostic factors in ALS. C-reactive protein (CRP) is a biomarker of the inflammatory response, and it shows significant prognostic value for several disease. To fully examine the prognostic significance of CRP in ALS we evaluated its serum levels at first evaluation in a large cohort of ALS patients followed from an Italian tertiary multidisciplinary centre. We replicated results in an independent cohort obtained from a population-based registry of ALS patients. We retrospectively enrolled 400 patients with a diagnosis of definite, probable and probable laboratory-supported ALS followed in a tertiary multidisciplinary centre. We replicated the analysis in an independent cohort consisting of 122 patients with ALS at different stages of the disease identified through a regional population-based registry. The results showed that CRP serum levels correlated with severity of functional impairment, as measured by ALSFRS-R total score, at first evaluation (r: -0.15297, p=0.0027), and with patient survival (HR 1.125 [1.058-1.196], p=0.0002), showing that ALS patients with high serum CRP levels at first evaluation were associated to a significant lower survival compared to those with normal serum CRP levels. Similar results were found in the independent cohort. Our findings confirm that CRP may be used as biomarker to stratify patients with a more prominent neuroinflammatory process possibly responding to targeted treatments.

P.03. Microvesicles and exosomes in ALS: biomarkers for disease propagation and therapeutic targets

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The lack of biomarkers in neurodegenerative diseases makes impossible to determine the stage of illness in patients delaying therapeutic trials. Blood contains extracellular vesicles (EVs), (mainly classified for size and biological function in exosomes-EXOs and microvesicles-MVs), pro-inflammatory vesicles that transfer mRNA, non-coding RNA (miRNA, LncRNA), and proteins among different cell types. A new hypothesis about disease transmission merges several neurological diseases associated with protein misfolding and aggregation, in the statement of "prion-like diseases". EVs can initiate prion propagation from prion-infected neuronal cells to uninfected cells, underlying a new mechanism of the disease propagation. The aim of our study is to investigate MVs and exosomes in plasma of Amyotrophic Lateral Sclerosis (ALS) patients, in order to discover a new mechanism in disease progression. Microvesicles and exosomes were isolated from plasma of 20 ALS, 20 healthy volunteers and 20 Alzheimer's

Disease (AD) patients. Dimension of MVs (200 nm-1 Î¼m) and EXOs (30 nm-130 nm) was confirmed by Nanosight. Markers for MVs of leukocyte (CD45), endothelial (CD31), platelet (CD61), erythrocyte (CD235a) derivation and apoptotic marker, Annexin V were investigated by flow cytometry. We found two groups of ALS patients: one with high CD45 derived blood MVs (6 fold more the healthy control group, p< 0,0001) and one with low CD45 MVs (0,5 fold less the healthy control group) (ANOVA test, p< 0,0001). SOD1, TDP43, FUS protein level was detected by WB in MVs and EXOs from ALS and healthy controls and then normalized against annexin V and Alix, respectively. Our preliminary data shows different misfolded protein level in EVs of patients compared to controls. EVs could have a relevant role in the disease propagation of ALS. Leukocyte derived MVs can be overexpressed in a group of ALS patients and they might be the "carriers" of misfolded proteins, main cause of disease propagation.

P.04. Mutated and non-mutated amyotrophic lateral sclerosis: a neurophysiological comparison

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INTRODUCTION: In the most of ALS patients we can't find a pathogenetic mutation, but in about 15% there are mutation in one of the known genes or loci described as covering a role in the disease. Clinical comparative studies between patients with different mutations have shown some phenotypic differences, but less is known about neurophysiological features. MATHERIAL AND METHODS: We investigated 40 ALS patient carrying mutations in one of the known ALS related genes (mtALS) with electromyography (EMG) and Motor evoked Potentials (MEP) and they were compared with 41 patients without any mutation in the most screened genes SOD1, FUS, C9ORF72, TARDP (stalls). For EMG we recorded ulnar, peroneal and median nerve cMAP, sural, ulnar and median nerve SAP and was performed needle examination. MEP were obtained stimulating the primary motor cortex and spinal roots, recording from upper limbs and lower limbs. We obtained the central conduction time (CCT)ad silent period (SP). We also calculated the neurophysiological index. RESULTS: Compared to wtALS, all mtALS patient have a greater EMG impairment of the abdominal muscles but in particular C9ORF, SOD1 and TARDP have less impairment of bulbar, cervical and lumbosacral districts. Optineurina showed greater signs of denervation and LMN degeneration compared to SALS. These data were confirmed by the analysis of NI. We found a greater cMAP reduction of Ulnar nerve (statistically significant) in SOD1 and TARDP compared to wtALS. Regarding MEP we found in mtALS a greater reduction of MEP amplitude and extended CCT compared to wtALS. This data is more evident in Optineurina subgroup and less evident in SOD1. CONCLUSIONS: We didn't find statistically significant neurophysiological alterations between mtALS and wtALS, however SOD1 mutations appear to be less seriously affected than other mtALS, especially with less bulbar impairment, perhaps in relation to the presence of the pseudopolyneuritic forms.

P.05. Metal concentrations in cerebrospinal fluid and blood plasma from patients with ALS

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A slow but steady increase in neurodegenerative disorders has been noted in recent decades. Degenerations in the nervous system are found in AlzheimerÂ´s disease, ParkinsonÂ´s disease and amyotrophic lateral sclerosis (ALS). In ALS spinal neurons degenerate causing muscle atrophy invariably leading to death from respiratory failure. What is causing this degeneration? No consistent explanation has been presented despite intense scientific efforts. ALS cerebrospinal fluid (CSF) studies (Karolinska Institutet Publishing http://hdl.handle.net/10616/41419) point towards metallotoxic etiologies. ALS clusters have been observed in regions with elevated metal concentrations in water and soil. Several studies show increased ALS incidence in certain occupations. ALS-like conditions are found in animals, notably in horses, where metal exposure can be suspected. Animal metal exposure experiments also show specific spinal cord accumulations of metals. To address possible metal contributions in ALS causation we measured concentrations of 22 metals in CSF from 17 ALS patients and 10 controls. Ethical approval was received. Statistically significantly increased concentrations were found for manganese (Mn), aluminum, cadmium, cobalt, copper, zinc, lead, vanadium and uranium. Manganese showed the most prominent differences between cases (median 5.67 mg/L) and controls (median 2.08 mg/L). CSF Mn concentrations were also higher than plasma Mn concentrations (median 0.91 mg/L), suggesting transport of Mn into the central nervous system in ALS patients. Most of the detected metals are neurotoxicants and synergistic effects can be anticipated. Properties of barrier systems will be discussed and the possibility of Mn accumulations and/or multimetal toxicity as a major factor contributing to the relentless course of ALS introduced.

P.06. Metal and proteomic analysis of sporadic ALS patients with common geographical origin

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Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder characterized by selective degeneration of both upper and lower motor neurons in the brain, brainstem, and spinal cord. This results in paralysis due to muscle weakness and atrophy, leading to death in 3-5 years. Genetic and environmental factors are involved in the pathogenesis of the disease and metals metabolism have been linked to ALS. This study enrolled seven patients and five controls (age matched, living in the same geographical area). For metal quantitation, samples of serum were analyzed by ICP-MS. For proteomic analyses, immobilized pH gradient covered the 4-10 and 3-7 pH range. Statistical analyses were carried out with Student's t-test and Artificial

Neural Networks. Among the metals analyzed, As concentration resulted significantly lower in patients than in controls (p=0.007); Hg too was found in lower concentration in patients, but with a lower statistical significance (p=0.13). Higher concentration of Al in patients was detected (p=0.08). In this study, we were not able to confirm the higher concentrations of Ni and Pb in patients previously described in a smaller cohort. Our proteomics data show that APOA2 is decreased by 30% in patients with respect to controls. Furthermore, AHSG and SAP showed a significant decrease in patients with a story of more than 10 years of disease. Impaired metal homeostasis, attributable to environmental exposure, could lead to mineral overload. Besides promoting oxidative stress, metals can compete for the binding sites of metal-containing proteins, such as those containing iron-sulfur clusters. At present, no literature data link APOA2 to ALS, but the fact that its mRNA is processed by TDP43, provides a possible connection with the disease. The proteins differentially expressed belong to the group of Acute Phase Reaction proteins, possibly linking ALS to a chronic inflammation status. Further experiments are still ongoing.

P.07. Gene expression biomarkers in ALS patients: a study in accesible samples

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Background: Previous studies performed on muscle biopsies from mice SOD1G93A suggested that this animal model presents an alteration in the expression of five genes (Mef2c, Gsr, Col19a, Calm1 and Snx10). Therefore the expression of those genes could behave like potential prognostic biomarkers of longevity [1]. Even though the search of biomarkers in ALS is being carried out in a wide variety of samples, growing tendency relies on the study of new and less invasive tissues [2, 3]. Our aim was to study the expression levels of MEF2C, GSR, COL19A, IMPA1, NOGOA and SNX10 in lymphocytes to obtain an association with disease progression. Methods: cDNA serial samples from lymphocytes of 45 patients with sporadic ALS, were subjected to qPCR in order to study expression levels of COL19A1, GSR, SNX10, MEF2C, IMPA1 and NOGO A, and then related with disease progression: ALSFRS-r, FVC and survival values. Patients were classified respect to their survival in 2 groups significantly different on a Kaplan Meyer study. Statistical analysis and ROC curves were made with GraphPad Prism software. Results: the expression levels of NOGOA and GSR were significant between patients and healthy controls (p value < 0,0001) related to survival, ALSFRS-r and FVC. ROC curves areas for GSR and NOGOA expressions, and the ratios GSR/NOGOA and NOGOA/GSR were respectively: 0,9939; 0,8788; 0,7867; 0,8049. Discussion: Gene expression levels of GSR and NOGOA, and the corresponding ratios, were found as valid diagnostic biomarkers of ALS. Acknowledgements This work was supported by grants PI12/03110, PI14/00088 and PI14/00947, from Instituto de Salud Carlos III (ISCIII). And the support of the Spanish Foundation for the development of ALS research (FUNDELA). References: 1. Calvo AC et al PLoS One 2012; 7:e32632. 2. Nachmany H et al. Disease Markers 2012; 32: 211-220. 3. Pradat PF et al. Ann. Neurol. 2007; 62: 15-20.

P.08. Early- and late-onset biomarkers in peripheral blood mononuclear cells of ALS patients

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One of the peculiar clinical characteristics of amyotrophic lateral sclerosis (ALS) is the wide distribution in age of onset, which has been reported to be associated with differing clinical presentations and prognosis. This study focuses on the identification of phenotypic protein biomarkers of ALS in patients with an early and a late disease onset aiming at the understanding of the mechanisms at the basis of the disease process and susceptibility. A differential proteomic analysis was performed in peripheral blood mononuclear cells (PBMC) from a group of 16 ALS patients with an age of onset below 55 years and a group of 16 ALS patients with an age of onset over 75 years, and age- and sex-matched healthy controls. We identified 42 differentially expressed proteins in the two groups of patients. Pathway analysis revealed that there was a significant enrichment in the "protein folding and stress response" functional category. We next validated a selected number of proteins belonging to this functional group on 84 patients and 76 age- and sex-matched healthy controls using immunoassays. The results of the validation study confirmed that there is an upregulation of proteins involved in protein folding in ALS patients with a late-onset. This may indicate that an upregulation of protective proteins, such as chaperones, may be at the basis of a different susceptibility of the disease in early and late-onset ALS. Moreover, we confirmed that total TDP-43 expression in PBMC is a marker of pathology, but cannot distinguish between the early- and the late-onset phenotype.

P.09. Clinical validation of pNfH and NfL as prognostic and diagnostic biomarkers for ALS

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Validated biomarkers are important for improving the early diagnosis of amyotrophic lateral sclerosis (ALS) as well as for clinical trials to stratify and monitor ALS patients. The most promising biomarkers are the neurofilaments, specifically the phosphorylated neurofilament heavy chain (pNfH) and the neurofilament light chain (NfL), which are major components of the motor neuron cytoskeleton. The aim of this study was to validate neurofilaments as diagnostic and prognostic biomarkers. We performed a two center study where we measured both pNfH and NfL concentrations in the cerebrospinal fluid (CSF) of 112 ALS patients and 225 neurological controls with commercially available ELISA assays (NfL: UmanDiangnostics AB, UD51001; pNfH: Biovendor, Brno, RD191138300R). Both pNfH and NfL concentrations were significantly increased in the CSF of ALS patients compared to neurological controls ($p<0.0001$). For CSF pNfH concentrations we acquired a cut-off value at 879.5 pg/mL with a sensitivity of 87.5% (CI 79.9 - 93.0%), a specificity of 88.8% (84.0 - 92.7%), a positive predictive value (PPV) of 79.7% (CI 71.5 - 86.4%) and a negative predictive value (NPV) of 92.9% (CI 88.3 - 96.0%). For CSF NfL concentrations an optimal cut-off of 2,867 pg/mL yielded a sensitivity of 80.4% (CI 71.8 - 87.3%), a specificity of 67.9% (CI 61.3 - 74.0%), a PPV of 56.3% (48.2 - 64.1%) and a NPV of 87.1% (81.1 - 91.7%). pNfH and NfL were significant higher in CSF of ALS patients with fast disease progression. Thus pNfH and NfL had added value as biomarkers in the diagnostic process for ALS and might predict disease progression.

P.10. Circulating exosomes as a novel source of biomarkers for ALS progression

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Exosomes are vesicles that fuse with plasma membrane and release its content extracellularly. They circulate in biological fluids and are involved in intercellular communication, carrying a variety of cargoes such as RNA, metabolites and proteins, including those prone to aggregation (e.g. SOD1 and TDP-43). We and others demonstrated that cells release exosomes constitutively, but increase their release in response to a variety of pathological conditions, including ALS, probably contributing to disease spreading and progression. Plasma concentration of exosomes and their biochemical properties are therefore accessible and measurable parameters that may underline disease progression. In this pilot study we tested the feasibility to use circulating exosomes as a source of biomarkers for ALS. We first optimized the protocol to isolate exosomes from plasma samples. Next, we verified the purity of the isolated fraction and characterized it through biochemical and physico-chemical analyses. Finally, we analyzed the level of a priori-selected candidate biomarkers, e.g. TDP-43 and SOD1, and performed an array of omics technologies in exosomes isolated from plasma of ALS patients and healthy controls. In conclusion, we demonstrated that circulating exosomes are a promising source of biomarkers that can be used in large clinical studies.

P.11. Blood Lead, Bone Turnover, and Survival in Amyotrophic Lateral Sclerosis

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Blood lead and bone turnover have been suggested to be associated with the risk of amyotrophic lateral sclerosis (ALS). We aimed to assess whether these factors were also associated with mortality after ALS diagnosis through a survival analysis among 145 ALS patients enrolled during 2007 in the National Registry of Veterans with ALS. Associations of mortality with blood lead and plasma biomarkers of bone resorption (C-terminal telopeptides of type I collagen; CTX) and bone formation (procollagen type I amino-terminal peptide; PINP) were estimated using Cox models adjusted for age at diagnosis, diagnostic certainty, diagnostic delay, site of onset, and the revised ALS functional rating scale. Hazard ratios (HRs) were calculated for each doubling of biomarker concentration. Blood lead, plasma CTX and plasma PINP were mutually adjusted for one another. Increased lead (HR=1.38; 95% confidence interval [CI]: 1.03, 1.84) and CTX (HR=2.03; 95% CI: 1.42, 2.89) were both associated with a higher mortality, whereas higher PINP was associated with a lower mortality (HR=0.59; 95% CI: 0.42, 0.83), after ALS diagnosis. No interactions were observed between lead or

bone turnover and other prognostic indicators. Lead toxicity and bone metabolism may be involved in ALS pathophysiology. Bone turnover biomarkers may be useful clinically in predicting ALS prognosis. Keywords: amyotrophic lateral sclerosis, blood lead, bone turnover, survival, veterans.

P.12. Alterations of Adiponectin in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Adiponectin (APN) is a major modulator of energy metabolism by enhancing insulin sensitivity coupled to its ability to stimulate mitochondrial biogenesis in skeletal muscle. Besides playing important roles at the interface between brain and adipose tissues, this adipokine appears strictly associated with both cerebrovascular and neurodegenerative diseases. In particular, APN has been described to be down-regulated in obesity as well as associated to Alzheimer's Disease (AD) where it correlates with severity of the dementia and predict AD onset. For this clinical study, a total of 112 subjects were enrolled, including 36 healthy controls (CTR), 8 Obese subjects (OB), 52 Amyotrophic Lateral Sclerosis (ALS), 6 Frontotemporal Dementia (FTD) and 10 AD patients. The cohort of ALS included both familiar and sporadic patients, as well as subjects with bulbar and spinal onset. All groups were age and gender matched, whenever possible, and the main metabolic alterations interfering with APN serum values were excluded. We analysed circulating APN levels by a specific diagnostic ELISA kit. We confirmed statistically lower APN mean values in OB and higher in AD patients. APN levels were significantly increased in FTD patients when compared to CTR with the highest values between all subgroups. Interestingly, the observed circulating APN levels were also significantly different between ALS and FTD patients, although these pathologies share highlight commonalities and overlapping features. Although mean serum APN values were comparable between all ALS and CTR, sex specific analysis enlightened an opposite trend in males characterized by statistically lower values, whereas female displayed significantly higher amounts than CTR of the corresponding sex. In conclusion, we observed highest circulating APN levels in FTD patients. Moreover, we demonstrated altered levels of APN in ALS patients compared to CTR, in a gender specific manner. Further investigations will clarify the possible involvement of APN in motor neuron disease.

Clinical aspects

P.13. The utility of multimodal imaging in the diagnosis of ALS

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Introduction. Advances in statistical learning theory were applied to assess the diagnostic potential of structural and diffusion tensor (DT) MRI in amyotrophic lateral sclerosis (ALS). Methods. 3D T1-weighted and DT MRI were obtained from 113 sporadic (probable, probable-laboratory supported, definite) ALS patients, 22 patients with ALS mimic disorders, and 40 healthy controls. The diagnostic accuracy of precentral cortical thickness measures and DT MRI metrics of the corticospinal tract and motor callosal fibers were assessed in a testing cohort and externally proved in a validation cohort using a random forest analysis. Results. In the testing set (64 randomly selected sporadic ALS patients and healthy controls), precentral cortical thickness showed 0.85 accuracy, 0.76 sensitivity, 1.00 specificity in differentiating ALS patients from healthy controls, while DT MRI measures distinguished the two groups with 0.77 accuracy, 0.84 sensitivity, 0.65 specificity. In the same group, the combination of cortical thickness and DT MRI metrics improved the classification pattern as follows: 0.87 accuracy, 0.88 sensitivity, 0.84 specificity. In the validation cohort (remaining 49 sporadic ALS vs ALS mimic disorders), the diagnostic accuracy was higher for DT MRI than cortical thickness measures (0.80 vs 0.65), and the combined approach improved the classification only minimally (accuracy 0.83). Conclusions. A multimodal imaging approach that incorporates motor cortical and white matter alterations yields statistically significant improvement in accuracy over using each modality independently in the individual ALS patient classification. DT MRI technique may be a useful tool in distinguishing ALS from ALS mimic disorders. Funded by: AriSLA (MacLearnALS Project).

P.14. The selective anatomical vulnerability of ALS – disease defining & disease defying brain regions

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Background: Neuroimaging in ALS has gained significant momentum in recent years highlighting ALS-specific pathology in early stage and presymptomatic disease. While imaging studies of ALS invariably highlight motor cortex, corpus callosum and corticospinal tract pathology, the characterisation of unaffected brain regions has become increasingly important to discriminate ALS from mimic conditions in classification studies. Objectives: The aim of this study is to highlight key basal ganglia nuclei, cortical grey matter regions and white matter tracts which are not affected by

ALS even in advanced disease. Methods: Ninety-four ALS patients and sixty-one age-matched healthy controls participated in a multi-parametric neuroimaging imaging study. Subcortical structures were evaluated based on high resolution 3D structural data sets, using intensity gradients and automated boundary corrections. Grey matter analyses were carried out using both cortical thickness analyses and voxel-based morphometry. Alterations in white matter integrity were assessed based on diffusion tensor imaging data using several diffusivity parameters. Results: Multi-parametric analyses revealed that the posterior occipital cortex is less likely to be affected in comparison to other brain regions. Similarly the bilateral post central gyrus is relatively spared in ALS. The commissural white matter tracts of the forceps minor, splenium of the corpus callosum and tracts remain relatively intact in ALS in sharp contrast to the striking degeneration of the genu and mid-body of the corpus callosum. Basal ganglia analyses also confirmed striking selective vulnerability, with the relative sparing of thalamic sensory nuclei, medial globus pallidus and amygdala. Conclusions: The systematic characterisation of unaffected brain regions in ALS has pragmatic implications to distinguish ALS from mimic disorders and "disease controls". Furthermore our findings raise important pathophysiological questions as to why certain brain regions remain relatively resilient to ALS pathology.

P.15. The construction of the Swedish MND quality registry

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Introduction In Sweden quality of care varies across ALS-teams. Our primary aim was to construct a national quality registry for ALS in Sweden, which will assure high quality care for all patients in an easily accessible way. Our secondary aim was to create a research platform based upon the registry, through prospective follow up of the entire community of ALS patients in Sweden. Method In February 2015 the Swedish MND registry was launched. The set of mandatory variables was decided through consensus in a steering group of 8 senior ALS physicians, including measurements of HAD, ALSFRS-R, BMI, spreading pattern, dysphagia- and hypoventilation-symptoms. A patient own reporting portal (PER) was also created, enabling the patients to answer questions about health and life status via Internet before meeting with the care providers. In a pilot effort, we included in the registry all patients that during 2013-2014 sought ALS care in any hospital of the Stockholm area (a population of 2.2 million). Results To date 224 ALS-patients from 13 different hospitals are included in the registry. From 2013-2014, 271 patients from Stockholm were registered for ALS care. Among them 52,8% were men and 47,2% women. The mean disease duration was 38,6 months (from symptom onset to death) and 21,7 months (from diagnosis to death). The largest age group was 60-69 years (32%). Patients at the age of 40-49 years and patients with spinal onset had longer disease duration compared to other patients. Discussion The Swedish MND registry is voluntary but easily accessible via Internet, and aims to aid the ALS clinicians as a

decision making tool and thus facilitates the clinical visit both for clinicians and ALS patients. By using PER, the patients become more involved in their own care and are given the opportunity to view a visual report of their status.

P.16. The ALS Stratification Challenge- Using big data to develop models for stratifying ALS patients

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Amyotrophic lateral sclerosis (ALS) shows significant heterogeneity in progression and survival. Patients show different disease development paths with highly different speeds of progression that make clinical trials planning and interpretation challenging, limit our ability to elucidate disease mechanisms and have led some to speculate that ALS might be an umbrella term for several distinct phenotypes. Therefore, understanding and sub-grouping the clinical manifestation of ALS is of interest. The DREAM ALS Stratification Prize4Life Challenge is a crowdsourcing initiative that aims to address the problem of ALS patient heterogeneity with regards to important clinical targets such as ALSFRS progression and survival. In the challenge, which ran on summer 2015, we asked participants to derive meaningful subgroups of ALS patients and predict disease progression and survival. The challenge used ALS clinical data from the PRO-ACT database, as well as data from National ALS registries from Italy and Ireland. The challenge drew in 288 participants and80 final submissions based on statistical and machine learning approaches. We used the clustering results of the algorithms developed as part of the challenge to detect patients which were consistently grouped together and identified four reliable clusters. These novel subgroups of patients had distinct clinical, physiological, progression and survival profiles. A few of the most distinctive features separating the clusters were symptoms onset location (limb/bulbar), time from disease onset, mobility and breathing measurements and disease progression rate. We will present a full description of the clusters and use the data and challenge outcomes from ALS national registries to demonstrate how our results transfer to the day to day patients in the clinical practice. These results demonstrate the value of large datasets and crowdsourcing challenges for developing a better understanding of ALS natural history, prognostic factors and for improving ALS clinical development.

P.17. Telesurveillance in ALS patients: a 3 year center experience

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ALS patients with respiratory failure use to be treated with home care non-invasive ventilation (NIV), or with invasive ventilation (IV) via tracheostomy. It is well demonstrate the role of home care in the management of motor neuron diseases. Telemedicine models can be useful to improve patients and caregivers autonomy, avoiding the therapeutic fractures from hospital to home. From April 2013 to March 2016, 74 ALS patients (64 NIV, 10 IV) resident in Piemonte and Valle d'Aosta who underwent NIV or IV were recruited; a cohort of 71 ALS patients from Piemonte and Valle d'Aosta Register (PARALS), matched for age, sex and presence of NIV or IV without telesurveillance service were considered as controls. The system consisted in a patient-dedicated tablet managed by a trained pneumologist who performed specialized home visits (when needed), connected with a provider call center for emergency calls; an oximeter for real time data and for monthly nocturnal oximetry. Each patient had to fill daily and fortnight questionnaires about his/her clinical status and mechanical ventilation parameters; according to the answers, telephone or video call with the pneumologist or home specialized visit or hospitalization were organized . 18545 daily clinical questionnaires (total adherence 58%; IV patients 65%; NIV patients 51%) and 1540 fortnight questionnaires (total adherence 76%; 77% IV; 80% NIV) have been fulfilled. During the first 18 months 15 unscheduled home visits and 113 phone contacts have been performed. 8 NIV patients needed hospitalization for a total of 99 days (3.1 days/pt/yr). Ten patients (33%) in the NIV group died. In the control group 8 cases needed hospitalization for a total of 132 days (5,5 days/pt/yr); their mortality was 33% (8 patients). Telesurveillance is an efficient system for home care management of ALS patients, in particular for costs cut and autonomy support. (Regione Piemonte Grant)

P.18. Survival of ALS patients is not affected by a psychosocial intervention in an ALS unit

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Introduction: Multidisciplinary care improves survival of ALS patients. The reasons for this positive effect remain unclear. Starting 2010, each patient is systematically evaluated by a psychologist and a social worker as part of the ALS unit team. Follow up is performed at each unit visit and at home according to each patient's own needs. This is in addition to standard care offered by a dedicated neurology, pneumology, nutrition and rehabilitation team. We sought to evaluate the possible effect of a psychosocial intervention on our ALS population. Results: We evaluated a total of 113 new consecutive patients since January 2010. We compared them with 196 patients from the period of 2007 - 2010. At baseline, patients in the psychosocial intervention group were of older age but otherwise comparable with the control group. On Kaplan Meier analysis mean survival for the intervention group was 39 months, with no significant difference with 41 month mean survival of the the control group (measured by log rank test, p 0.62). Conclusion: A psychosocial intervention did not

modify the survival of ALS patients treated in our ALS unit. This intervention must be understood as a tool for the improvement of social functioning and mental health well being. Further research is needed to better define which variables determine the survival benefit of ALS units.

P.19. Structural brain MRI abnormalities in Kennedy's disease

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Introduction. Diagnosis of Kennedy's disease (KD) might be challenging, as patients are often misdiagnosed as amyotrophic lateral sclerosis (ALS). Subtle MRI alterations have been reported in KD, but the extent of central nervous system involvement relative to ALS still needs to be investigated. We investigated cortical and white matter (WM) alterations in a large sample of KD patients compared to healthy subjects and ALS patients. Methods. 19 patients with genetically confirmed KD were compared with 21 controls and 17 sporadic ALS patients matched for demographics and disease severity. All patients underwent clinical assessment and MRI. Tract-based spatial statistics was applied to investigate WM damage, and cortical thickness analysis to identify cortical atrophy. Results. KD patients were characterized by pronounced behavioral symptoms and only subtle cognitive deficits. Relative to controls, KD patients showed severe damage of the pontine crossing fibers, right frontotemporal and fronto-occipital tracts, right cingulum. They also showed subtle cortical thinning of the inferior frontal gyrus bilaterally, left premotor regions, middle temporal gyrus, and right precuneus. ALS patients, compared to controls, showed the classic pattern of damage of the corticospinal tracts (CST) and corona radiata bilaterally with an additional involvement of the left superior longitudinal (SLF), frontotemporal and fronto-occipital tracts, and cortical thinning of precentral gyrus, frontal cortex, lateral temporal and parietal regions, and precuneus bilaterally. The involvement of the CST, corpus callosum, external capsule bilaterally, and left SLF was greater in ALS compared with KD. Conclusions. We found subtle cortical abnormalities and the involvement of long-range frontal and limbic connections in KD patients, probably related to their behavioral abnormalities. The pattern of WM damage is similar in KD and ALS, while diffusion tensor MRI measures of the CST and corpus callosum represent powerful tools to differentiate ALS from mimic syndromes, including KD. Funding. Italian Ministry of Health (#RF-2010-2313220).

P.20. Sialorrheia and reversals in ALSFRS

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Introduction Sialorrhea is a debilitating symptom in bulbar-ALS patients, related to dysphagia, weak lips and drop neck. Its pharmacologic treatment has a potential implication on functional assessment, which we aimed to approach. Methods We included consecutive ALS patients followed in our Unit. Patients with other disorders were excluded. ALS functional rating scale (ALSFRS), bulbar subscore (ALSFRSb) and 1st (Q1), 2nd (Q2) and 3rd (Q3) question scores were recorded at study entry (T0), after 3 (T1) and 6 months (T2). A smaller group of patients were evaluated 9 months after entry (T3). Paired t-test was used to compare decay-rate between different time-frames (T0-T1, T1-T2, T2-T3). Pearson's test assessed correlations between variables. Regression model was applied to test if Q1, Q2 and Q3 declined independently from total ALSFRSb's decay. A $p<0.05$ was considered significant. Results We included 533 patients (295 men; mean onset age: 61.3±12; mean disease duration: 17.3±21.6months), 352-spinal, 158-bulbar, 27-respiratory/axial-onset. For each time-period all variables decayed significantly and were correlated between them ($p<0.001$). ALSFRSb was significantly dependent on Q1, Q2, Q3 in all periods ($p<0.001$). In 40 patients (7.5%) ALSFRSb, Q1, Q2 and Q3 ($p<0.001$, $p=0.006$; $p<0.001$; $p=0.003$) increased significantly between T0-T1, with no ALSFRS decline ($p=0.186$). Between T1-T2, ALSFRSb, Q2 and Q3 ($p<0.001$, $p<0.001$, $p=0.038$) increased significantly in 49 patients (9.2%), but not for Q1 ($p=0.133$), with a mild ALSFRS decline ($p=0.039$). Between T2-T3, ALSFRSb and Q2 increased significantly ($p<0.001$), not observed in Q1 and Q3, and ALSFRS remained stable ($p>0.05$). All patients with sialorrhea were treated for this symptom. Conclusion Individual bulbar questions were well correlated between them and with ALSFRSb. All variables declined significantly in 3-month periods in the total population. In slow progressors sialorrhea treatment could impact on ALSFRSb decline, which should be considered in clinical trials.

P.21. Selective white matter vulnerability in ALS: implications for diagnostic classification

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Introduction Magnetic resonance diffusivity measures have been repeatedly proposed as biomarkers of neurodegeneration in ALS, but no consensus exists as to which diffusivity parameter or white matter segment is most sensitive to neurodegenerative changes. The objective of this study is the in depth characterisation of white matter pathology in ALS in order to differentiate patients from controls. Methods A large neuroimaging study has been undertaken with 62 ALS patients and 55 age-matched healthy controls. Tract-based white matter alterations were explored based on fractional anisotropy (FA), radial- (RD), mean- (MD), and axial diffusivity (AD) indices. The corticospinal tract was segmented into the lateral fibres of the corona radiata, medial corona radiata, internal capsule, cerebral peduncles and corpus callosum into the genu, body and splenium. First, the average percentage change of each segment was calculated. Subsequently, a multiple binary regression was carried out for each diffusivity measure to identify the most discriminating segment. The best subset was selected based on Bayesian information criterion. Results RD reflected the most pronounced percentage change. The most severely affected region was the body of the corpus callosum where patients exhibited a 7.62%

change in RD, 3.94% in FA, 2.89% in MD, followed by the cerebral peduncles where patients displayed a 5.98% change in RD, 3.3% in FA, 1.8% in MD. The best model differentiating patients and controls based on FA or RD alterations identified only one region: the cerebral peduncles. Based on MD, the model included the cerebral peduncle and the body of the corpus callosum. Conclusions ALS patients and controls can be best discriminated based on the radial diffusivity changes of the cerebral peduncles. Our results have important implications for future classifier studies; identifying RD as the most sensitive imaging marker and the cerebral peduncles as a candidate target brain region for diagnostic classification models.

P.22. Ptosis and bulbar-onset in familial ALS: two different diseases or a new clinical phenotype?

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Ptosis isn't a feature observed in Amyotrophic Lateral Sclerosis (ALS). In literature were reported only one patient with ALS and generalized myasthenia gravis, two bulbar-onset ALS patients with fluctuating ptosis and diplopia and two with increasing of anti-AchR antibodies (without clinical myasthenic features). We describe a case of familial bulbar-onset ALS with ptosis manifested at the onset of the disease. Case reports: we describe the case of two siblings,a 70-year-old woman and a 72-year-old man, who presented to our tertiary ALS Centre because of a progressive bilateral ptosis followed by dysarthria and dysphagia. Clinical examination showed, in both cases, proximal upper limb muscular weakness with fasciculations, hyperreflexia at lower limb and a bilateral symmetric ptosis (without diplopia). Laboratory tests (including anti-AchR, anti-MUSK and anti-LRP4 antibodies), brain MRI and repetitive nerve stimulation (in nasal muscles and in abductor digiti quinti) were normal. EMG revealed diffuse fasciculations. Thymoma was excluded by TC. In man muscle biopsy (left brachial biceps) was made showing only denervation. Neuropsychological evaluation showed deficits in visuospatial/attentive domains. In both patients we observed a progressive clinical decline and a definite ALS's diagnosis according to the El Escorial criteria were made approximately one year after the onset. No mutations in FUS, TARDBP, SOD1 and C9orf72 genes. The woman died 5 years after the onset. The man, after one year from the diagnosis, needs NIV support during the night. Discussion: this's the first report of familial ALS with bilateral ptosis at the onset. An association between ALS and ocular myasthenia were excluded because anti-AchR antibodies were negative, no thymoma was detected and neurophysiological tests showed no neuromuscular transmission failure. The rapid course of disease, and the absence of systemic involvement, excluded a mitochondrial disease. We think that our patients represent a new clinical phenotype of familial ALS with unknown gene.

P.23. Premorbid body mass index and its behavior during follow up is a predictor of survival in ALS

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Nutritional status is a prognostic marker at the time of diagnosis. We prospectively describe the nutritional status of first evaluated patients on a multidisciplinary care clinic, evaluating its relationship with survival. We obtained data on premorbid weight, BMI at first visit and 6 months of follow up. We evaluated a total of 221 patients between January 1st, 2010 and January 1st, 2015. Results: 146 patients died during follow up (66,1%). The mean survival time was 40 months. Bulbar patients had a shorter survival time (35 months vs 39 p = 0,04). Age over 60 was also related with shorter life span (36,8 vs. 69,3 months, p = 0,006). Mean BMI at first visit was 25,5 kg/m2. Just 11 patients were malnourished while 30 patients where clinically obese at the time of diagnosis. Survival time was longer for overweight patients, with a mean of 49 months, which reduced to 38 months for obese patients and only 32 months for normal weight individuals (p = 0,007). 57 patients had a weight loss of over 10% at diagnosis. For these patients, survival time was reduced to 28 months (p < 0,001). While weight loss was more frequent in bulbar patients (39,6% vs 21,4%) spinal patients also presented weight loss frequently and related with worse prognosis (mean survival time of 27 months). Follow up data was available for 192 patients. 15 patients had further weight loss, again related with worse prognosis (survival 29 months, p = 0,009). The use of gastrostomy did not influence survival time independently of bulbar onset or weight loss.

P.24. Predictors of adaptation to non-invasive ventilation in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a multisystemic disease compromising both the neuromuscular system and the cognitive status. The cognitive impairment varies from an isolated executive dysfunction to a severe frontotemporal dementia. Respiratory failure is the most frequent cause of death in patients with ALS. Non-invasive ventilation (NIV) has been shown to improve survival and quality of life in ALS patients with respiratory insufficiency, but scanty literature investigated which are the predictors of NIV tolerance. The aim of this study was to evaluate the impact of functional, cognitive and neurobehavioural and respiratory status on NIV tolerance in patients with ALS. We retrospectively evaluated clinical data of ALS patients who underwent a NIV starting training during hospitalization over a year. Cognitive and neurobehavioral assessments have been performed using the Edimburgh Cognitive and behavioural ALS screen (ECAS), the Hospital Anxiety and Depression Scale (HADS), the Frontal Assessment Battery (FAB), the Progressive Matrices 47 (PM47) and the Neurobehavioral Rating Scale - Revised (NRS-R). Seventy-two patients (mean age: 63.9 yrs±10.65SD) were included. Of these 49 were non bulbar ALS (with different phenotypic variants) and 23 were bulbar phenotypes. The time required to reach a satisfying NIV adaptation significantly related at multivariate analysis to presence of sialorrhea (r 0.30, p=0.028), respiratory status (Borg Dyspnoea Scale, r -0.33, p=0.006 and ALS-FRS-R respiratory subscore, r 0.30, p=0.033) and behavioural and cognitive impairment (PM 47, r -0.20, p =0.043, NRSR-F1, r 0.20, p=0.044, NRSR- F5, r 0.24, p=0.048). Our study showed that presence of sialorrhea, the level of respiratory symptoms, neurobehavioral impairment are negative predictors of NIV adaptation. This study highlights the need of a multidisciplinary patient-tailored approach including psychological supportive care to optimize patient's training and compliance to NIV.

P.25. Predicting Survival of ALS Patients from Diffusion-Weighted Images Using Deep Learning

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Reliable prediction of survival, highly variable in amyotrophic lateral sclerosis (ALS), at the time of diagnosis would be invaluable for ALS patients and patient caregivers. Clinical characteristics contain useful information for survival prognosis but this prognostication remains too uncertain to provide in clinical practice. Neuroimaging data capture (disease-related) brain characteristics which provide additional prognostic value. In this study, clinical characteristics are combined with structural connectivity MRI to predict survival of ALS patients using deep learning, a machine learning technique shown to be very useful in big-data classification problems. A large group of 135 ALS patients was included of which high-resolution diffusion-weighted images were acquired at first examination. Furthermore, clinical characteristics including site and age of onset, time to diagnosis, ALSFRS slope, FVC, C9orf72 status, FTD status and El Escorial criteria were recorded and their survival time from disease onset to death was recorded. Patients were labeled as short, medium or long survivors according to their survival time, creating a classification problem for deep learning to be solved. From the total group of 135 patients, a training set for deep learning (n = 83 patients) was selected. A validation set (n = 20) was used to prevent deep learning from overfitting. Finally, an evaluation set (n=32) assessed the performance of the obtained deep learning networks. Deep learning on clinical characteristics and structural connectivity MRI alone yielded correct predictions of survival class in 68.2% and 63.6% of the cases, respectively. Importantly, the combination of these two sources of information significantly increased deep learning accuracy to 77.3%. Taken together, our findings show that by combining clinical characteristics and baseline MRI data correct survival class can be predicted for 3 out 4 cases, demonstrating the benefits of deep learning strategies in disease prognostication.

P.26. Phenotype comparison between young- and elderly-onset motor neuron disease patients

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INTRODUCTION. Literature suggests that motor neuron disease (MND) has wide phenotypic heterogeneity. We explored differences between young- and elderly-onset MND patients. METHODS. Within our cohort of 752 MND patients, we compared phenotype between the 5% with the youngest age at onset (AAO) ("young patients") and the 5% with the oldest AAO ("elderly patients"). RESULTS. Of the 752 patients (480 males and 272 females), 89.4% had ALS, 5% had primary lateral sclerosis (PLS), and 5.6% had progressive muscular atrophy (PMA). Mean AAO was 58.3 years. Site of onset was spinal in 76.3% and bulbar in 23.7%. Young patients (n = 38, mean AAO 30.4 years, range 18-37) were predominantly males (76.3%), while elderly patients (n = 38, mean AAO 79.7 years, range 77-87) were predominantly females (55.3%, p = 0.009). Bulbar onset was significantly more frequent in the elderly (16 = 42.1%) than in the young (5 = 13.2%, p = 0.009). Young patients more frequently displayed a pyramidal phenotype (16 = 42.1% vs 4 = 10.5% among the elderly, p = 0.003) and had longer survival (mean, 103.5 vs 26.3 months). Mutations in ALS genes (FUS, SOD1, TARDBP, UBQLN2, CHCHD10) were found in 8 (21.1%) young patients but not in the old. CONCLUSION. Our study shows that young- and elderly-onset MND patients are phenotypically different, with young-onset cases displaying a limb-predominant and pyramidal phenotype and longer survival. Young-onset patients also carry a heavier genetic burden.

P.27. Perceived Assistance Questionnaire is a useful tool for the self assessment of quality of care providers

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The implementation of multidisciplinary clinics has improved survival and quality of life in ALS. There is still a lack of consensus on what standard and which measures of quality of care these units should achieve. We sought to evaluate the degree of satisfaction of patients with ALS (PALS) with the current care provided by our ALS unit. Patients were asked to rate the self perceived quality of care through a survey of the services provided by UFELA (Unitat Funcional de ELA). Three mayor aspects were valued: Accesibility to the service, quality of the information received and quality of care. A total of 72 patients in different stages of the disease responded to the survey. 48,6% were male. The average age of the population was 66 years. Waiting times for a visit were judged as short by 52,8% of patients. Only 6,9% of patients considered the time to get a visit as excessive. On the day of the clinic visit, most patients described the time in the waiting room as fair (58,3%) or short (26,4%), and the number of people at the waiting room as small (8,3%) or moderate (70%). 37% of the patients had made telephone inquiries. Information on the disease was rated as

good in both quality and quantity in the vast majority of cases (80,6%) We also rated information give on additional tests performed and their results which was rated as very good (79- 82%). Patients gave special value to information on social resources which was classified as very good in 89,4%.of cases. Only 40% of patients knew the advance directive possibility and only 12,5% had the document. An additional 35% showed interest in more information. They valued positively the respect for privacy and participation in decision-making and the team's ability to respond psychological needs. Good or very good in 80 and 85% of cases. Self assessment is an important tool to ensure that the quality of care provided by the ALS clinic is maintained. Consensus is necessary among ALS units across Europe on the best way to homogenize services offered to PALS and monitor its standards.

P.28. PLS-FTLD, expanding the spectrum of dementia in motor neuron disease

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Primary lateral sclerosis (PLS) is a rare motor neuron disease, characterized by the exclusive degeneration of upper motor neurons leading to slowly progressive spasticity. In contrast to ALS, where cognitive impairment and frontotemporal dementia (FTD) are frequently seen, relatively little is known about cognition in PLS. The objective of this study is to report the clinical findings and frequency of PLS patients that developed FTD in a referral-based cohort and provide a review of the literature. Six out of 180 (3.3%) PLS patients developed FTD. All patients showed slow motor deterioration over the course of many years (average 7 years) before developing rapidly progressive behavioral changes and language deficits. The average time between the first cognitive changes and the diagnosis of FTD was 6 months. Subclinical cognitive deficits have reported in PLS, but to date only 7 cases of PLS and FTD have been reported in the literature. We demonstrate that PLS patients may develop FTD and that perhaps motor decline and cognitive deterioration occur at different speeds in motor neuron disease. Moreover, our data suggests that FTD in PLS patients is underreported. Literature and this report suggest the cognitive profile in PLS to be similar to ALS (within the spectrum of FTD), which raises the question whether PLS is a slow form of ALS.

P.29. Motor neuron disease with very long disease duration or CMT?

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SOD1 mutations accounts for 20% of familiar cases but can be found in sporadic cases of ALS. Some of them are known to induce peculiar slow progressing phenotypes. A 75 years old man was referred to our clinic for suspected motor neuron disease (MND). His family history was negative for neuromuscular diseases and past medical history was not significant. Symptoms started at the age of 24 and were characterized by feet numbness. At age 30, the patient started having progressive distal muscular weakness localized in the lower limbs which than extended proximally and later involved also upper limbs. From 2000 he has been walking with aide and in the past 3 years he has started using a wheelchair. Bulbar and respiratory symptoms were absent. As he first came to our attention (2007), he presented important distal weakness and moderate proximal weakness in the lower limbs associated with bilateral calves atrophy. Tendon reflexes were absent in the lower limbs. He didn't show any clinical sign of upper motor neuron involvement. EMG showed axonal motor polyneuropathy accompanied by moderate sensory involvement. The patient underwent genetic screening for MND which revealed a heterozygous pathogenic mutation in SOD1 gene (c64G>C/ p.Glu22Gln, exon 2) which has been described as causing slow progressing ALS phenotypes. However the exceptionally long disease duration (46 years), prolonged isolated distal lower limbs involvement and sensory symptoms may also fit with Charcot Marie Tooth disease (CMT) phenotype. Our observation raises questions about the definition of clinical spectra of ALS and CMT as already seen in VCP gene mutations, which have been associated both with ALS and CMT2Y. As described, the distinction between these pathologies can be blurred. It is anyway of primary importance to reach a genetic definition for correct counselling and follow up of patients and their families.

P.30. Motor Unit Number index (MUNIX) of six muscles: Normal values and effects of age and gender

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Introduction (background and aims): Motor Unit Number index (MUNIX) is a noninvasive method that requires minimal electrical stimulation. The technique involves utilizing the surface-recorded compound muscle action potential (CMAP) and electromyographic (EMG) interference pattern to compute the motor unit number index (MUNIX). The aim of this study is to establish MUNIX normative data profile of controls and to determine the degree of variance the effect of gender, and whether MUNIX declines in normal ageing. Methods: We have performed MUNIX in a cohort of 40 healthy controls to determine the normative range, variance and effects of gender and ageing on the outcome. Results: In normal subjects MUNIX decreased

slightly with age and showed excellent reproducibility. There was no gender effect and a highly significant correlation was found between MUNIX values and CMAP amplitude. Conclusion: Our study established MUNIX data profile of a cohort of normal controls and determined the degree of variance as well as the effects of gender and age. Keywords: MUNIX, Normative data.

P.31. Longitudinal recordings of eye movements confirm sequential oculomotor alterations in ALS

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Background: Recordings of eye movements have demonstrated that oculomotor dysfunction in patients with amyotrophic lateral sclerosis (ALS) follows a two-staged pattern [1] that is consistent with the model of sequential corticofugal axonal spread of pTDP-43 pathology [2]. We aimed to investigate whether this model can be confirmed in a larger cohort (N=199) and in longitudinal testing. Methods: Eye movement recordings using video-oculography (EyeSeeCamÂ®) together with clinical (ALS-FRS) and neuropsychological scores (ECAS) from 199 ALS patients and 31 matched healthy controls were obtained in a longitudinal approach. Results: At first investigation, 75 patients presented no oculomotor deficits ('nd') compared with controls. 87 patients showed stage 1 symptoms with only executive dysfunction and 37 patients showed stage 2 pathology with additional cerebellar type of smooth pursuit and/or gaze palsy. Seven patients with no deficits at timepoint 1 developed only executive dysfunctions (nd =>stage 1) and two patients (categorized as stage 1 at timepoint 1) developed to stage 2, accompanied by a gradual worsening of executive functions. The oculomotor stages were significantly correlated ($p<0.001$) with both, the ALS-FRS, and the ECAS total score. Conclusion: The model of a two-staged sequential pattern of eye movement abnormalities in ALS could be confirmed by a lager cohort. Additional preliminary longitudinal data in 25 patients showed a progression from stage 0 to 1 or from 1 to 2 respectively. Thus our data favor the hypothesis that oculomotor decline in ALS depicts the neuropathological sequential spreading of the disease instead of being the consequence of different phenotypes of ALS. Oculomotor staging may serve as a technical marker of the neuropathological disease progression in correlation to the clinical phenotype. References: 1. Gorges M, Müller H-P, Lulé D, et al. (2015). PLoS One 10:e0142546. 2. Brettschneider J, Del Tredici K, Toledo JB, et al. (2013). Ann Neurol 74:20-38.

P.32. Longitudinal characteristics of latent ALSFRS-R subscores

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Introduction: Latent multi-dimensionality has been identified in the ALSFRS-R score

with sub-scores corresponding to motor, bulbar and respiratory dimensions. We explored the characteristics of this multi-dimensionality in longitudinal models. Methods: All cases with ALSFRS-R scores on the Irish ALS register were included. A linear mixed effects multi-level model was used to account for repeated measures of ALSFRS-R within individuals. Site of onset was added to the model as a fixed effect, first as an independent covariate, and then as an effect modifier. Model fits were compared via ANOVA. The final model for total ALSFRS-R was then altered to consider individual ALSFRS-R sub-scores as the outcome variable Results: 408 cases were identified with valid ALSFRS-R scores - 234 (57%) were male, 125 (31%) had bulbar onset disease. Longitudinal models revealed that the ALSFRS-R motor sub-score deteriorated earlier but at a similar rate in spinal onset disease, whilst in bulbar onset disease the ALSFRS-R bulbar sub-score deteriorated earlier and faster than in spinal onset disease. Graphs of model fit indicated that there was some sub-score decline before the reported date of onset. Discussion: The results are congruent with the observation by others that the ALSFRS-R should be considered as an aggregate of scores from three separate domains rather than a single metric. Our analysis indicates that the deterioration of ALSFRS-R sub-scores differs between spinal and bulbar onset patients. Models also indicate that ALSFRS-R sub-scores decline may begin before the reported date of onset.

P.33. Longitudinal Effects of Mindfulness on People with Amyotrophic Lateral Sclerosis and their Caregiver

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Introduction: Mindfulness is the process of actively making new distinctions, rather than relying on habitual or automatic categorizations from the past. Mindfulness has been positively associated with physical well-being, better recovery rates from disease or infections, pain reduction and overall quality of life (QOL). Thus far, the aims of psychological studies on ALS have focused on understanding patient (pALS) - and, to a lesser extent, caregiver - QOL and psychological well-being. No previous study has investigated the influence of psychological factors on ALS. Methods: A sample of 197 pALS, together with their caregivers (n=114), were recruited and assessed online twice, with a duration of four months between the two assessments. Assessments included measurements of trait mindfulness, physical impairment, QOL, anxiety and depression, as well as caregiver burden. The influence of mindfulness as predictor of changes in psychological and physical outcomes was evaluated with a mixed-effects model. Results: Mindfulness predicted higher QOL and psychological well-being, lower depression and anxiety, for both pALS and caregivers. It also predicted lower burden in caregivers. Interestingly, mindfulness positively influenced the change of physical symptoms: subjects with higher mindfulness experienced a slower progression of the disease after four months. Conclusions: These results showed that mindfulness is positively related to quality of life and well-being of both pALS and their caregivers. Furthermore, it appears that mindfulness resulted associated with a reduction of the disease progression. Possible clinical implications could be highly relevant for ALS care.

P.34. Is firstly diagnosed ALS really ALS? Results of a population-based study with long-term follow-up

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While misdiagnosis of ALS is not uncommon in the early stage of the disease, a false-positive diagnosis has been poorly investigated. Diagnosing ALS in a patient with another clinical condition not only exposes him/her to inappropriate treatments, but also prevents the use of more correct therapeutic interventions and has relevant ethical and emotional implications. We reviewed the first diagnosis in a population-based incident cohort of patients with ALS undergoing long-term follow-up to investigate the long-term survival of the disease. The study population was represented by all the residents of nine administrative districts of Lombardy, a 23,851-km2 area of Northern Italy hosting a population-based ALS registry since January 1 1998. To determine long-term survival, the cohort was followed prospectively for 1,754 person-years. During follow-up, the caring neurologists were asked to confirm the diagnosis made at registration and, if the case was not confirmed with ALS, to give the alternative diagnosis. The study cohort included 496 eligible cases who were registered during a 5-year period (1998-2002). Included were 280 men and 203 women aged 18 through 93 years. During follow-up, 27 cases received a diagnosis different from motor neuron disease. These included spondylotic myelopathy (6), polyneuropathy (6), pseudobulbar palsy (4), spinal muscular atrophy (2), multiple sclerosis (2), other (5), unspecified (2). Two additional patients had progressive muscular atrophy and one had primary lateral sclerosis. The results of this and other studies tend to confirm that a first ALS diagnosis can be changed in about 6% of cases during follow-up. In the absence of diagnostic markers, each patient should know that at the time of a first diagnosis, the possibility exists that another, less severe clinical condition, may be present.

P.35. Increased risk of ALS for frontline workers

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Introduction: ALS has been associated with occupational and environmental exposures but until now findings have been inconsistent. Aim of this study was to assess whether previous employment in certain professions could be a risk factor for ALS occurrence. Methods: The study population consisted of all subjects over 15 years old who worked or were unemployed at 1991 Italian census and resident in Turin up to 1996 (n=324,464), followed up for ALS occurrence from 1996 to 2014. The risk of ALS was estimated in relation to the last profession held in 1991, for all professions with at least 5 exposed cases observed during the follow up, using the Italian National of Statistics classification of professions at the greatest detail (4 digits). The association between each profession and ALS risk was estimated through Poisson

regression models adjusted for age, gender, education and marital status. Results: During the follow-up 208 employers developed ALS, 70% men and 30% women. ALS risk was significantly associated with previous employment as bank tellers (IRR=7.74), general practitioners (IRR=4.76) and sales representatives (IRR=3.24). Given that a common feature of these professions is the direct contact with patients and costumers, we categorized all professions as exposed or unexposed to direct contact with the general public, such as patients, customers and pupils. It was found that previous employment in professions involving direct contact with general public increased significantly ALS risk (IRR=1.51). Conclusions: The study results indicate that ALS clusters in subjects employed in professions implying direct contact with the general public, suggesting that frequent exposure to people outside of the work organization may increase ALS risk. A possible explanation of this finding, partly supported from the literature, is that workers in contact with the public could be more exposed to certain infections, which in turn would increase their ALS risk.

P.36. Impairment of sensory-motor integration at spinal level in amyotrophic lateral sclerosis

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Objective: Subclinical sensory defect can be detected early in ALS. Its impact on spinal excitability was assessed by testing the effects produced by intrinsic hand muscle afferents in triceps brachii motoneurons of patients with distal motor weakness. Methods: TMS was applied over the motor cortex to produce MEP in contralateral triceps during tonic contraction. The intensity varied to compare the full MEP recruitment curve in ALS patients and controls. Then, median and ulnar nerve stimulations at wrist level were combined to TMS to compare the resulting changes in MEP size in both groups. Results: MEP recruitment curves were similar in both groups but MEP threshold was significantly higher in ALS. At sub-threshold intensity for MEP, TMS depressed more EMG activity in ALS than in controls. Nerve stimuli increased MEP size in both groups with similar temporal characteristics but the level of facilitation was stronger in ALS. Conclusion: Cortical hypo-excitability in ALS was accompanied with stronger intra-cortical inhibition in triceps area. While the corticospinal and peripheral inputs were likely depressed, spinal motoneuron response to combined inputs was particularly enhanced in ALS. Significance: Spinal network properties likely compensate for depression of afferent inputs leading to motoneuron hyper-excitability, which may contribute to excito-toxicity.

P.37. Hypothalamic dysfunctions in Amyotrophic Lateral Sclerosis

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Motor symptoms of Amyotrophic Lateral Sclerosis (ALS) are frequently accompanied by weight loss. ALS-related weight loss is an early phenomenon, occuring before onset of motor symptoms in patients, and is accelerated by the progression of bulbar symptoms. Weight loss is also found in animal models of ALS, and counteracting it through high caloric intake delays motor neuron degeneration. In patients, weight loss is correlated with survival, and a first clinical trial in gastrostomized ALS patients suggested protective effect of high caloric intake. Despite convincing evidence of the importance of weight loss in ALS disease progression, its underlying mechanisms are unknown. Body weight is controlled by a balance between food intake and energy expenditure integrated through a complex network of neuropeptides in the hypothalamus. Here, we will describe our recent efforts in characterizing hypothalamic function and dysfunction in ALS patients and mouse models. We have combined clinical, imaging and pathological studies in patients to obtain multiple evidences of abnormalities in hypothalamic function in ALS patients. We further delineated a subset of hypothalamic nuclei, and hypothalamic neuropeptides, particularly involved in ALS. In particular, we obtained indirect evidence of increased melanocortin tone in ALS patients. Most of these results were confirmed in various mouse models of ALS, including SOD1, FUS and TDP43 mice. Our combined results suggest distinct neural pathways underlying weight loss and abnormal energy intake behavior. In all, our studies identify druggable pathways to treat weight loss in ALS patients besides nutritional interventions. References: Vercruysse P, Sinniger J, El Oussini H, Scekic-Zahirovic J, Dieterlé S, Dengler R, Meyer T, Zierz S, Kassubek J, Fischer W, Dreyhaupt J, Grehl T, Hermann A, Grosskreutz J, Witting A, Van Den Bosch L, Spreux-Varoquaux O, Ludolph A.C. & Dupuis L. Alterations in the hypothalamic melanocortin pathway in amyotrophic lateral sclerosis, Brain. 2016, in press

P.38. Gray matter changes and pseudobulbar affect in patients with ALS: a voxel-based morphometry study

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Background: Pathological lauging and crying (i.e. pseudobulbar affect [PBA]) has been traditionally associated with damage to the cerebro-ponto-cerebellar pathways arising in the motor areas and descending to the brainstem and cerebellum, with anterior and posterior associative areas being also implicated. Although the disruption of reciprocal pathways between cortical and subcortical areas has recently been studied using advanced neuroimaging techniques, the pattern of ALS cortical changes in relation to PBA has not been thoroughly examined. Aim: To investigate gray matter volume (GMV) changes in non-demented ALS patients with or without PBA using

whole-brain voxel-based morphometry (VBM) analysis. Methods: Thirty-six patients with ALS diagnosed according to the revised El-Escorial criteria and 25 healthy controls (HC) were included and scanned on a 3T-Philips Achieva-Tx MR-scanner acquiring high-resolution 3D-T1-weighted anatomical dataset. Pathological laughing and crying was assessed using the Centre for Neurological Study-Lability Scale (CNSLS) with a cut-off score of â‰¥13 for PBA. Whole-brain VBM analysis was conducted to examine GMV changes between patients with and without PBA and HC ($p<0.001$ uncorrected; extent threshold: expected voxels per cluster). Results: Compared to HC, patients without PBA showed decreased GMV in right precentral gyrus and anterior cingulate gyrus (ACC), left cuneus and superior temporal gyrus, whereas patients with PBA showed more widespread GMV changes in frontal (inferior orbitofrontal; rectus); temporal (superior); occipital (cuneus; fusiform; lingual gyrus) regions, ACC and posterior cerebellum bilaterally. None of the groups had increased GMV compared to HC. Patients with PBA also presented decreased GMV in right precuneus/posterior cingulate cortex and middle temporal gyrus compared to patients without PBA. Conclusions: Whole-brain volumetric analysis may reveal specific GMV alterations in ALS patients with and without PBA. The pattern of GMV changes in patients with PBA is discussed under the view of ALS-related emotional perceptual problems often associated with right hemispheric dysfunction.

P.39. Functional and structural disruption of transcallosal pathways in ALS: a DTI-TMS study

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Background: Recent imaging studies showed that the corpus callosum (CC) is affected in ALS (Müller et al., 2012). Also, some studies suggest that interhemispheric connectivity is altered on a clinical level, as involuntary mirror movements (MM) during unilateral hand movements were described in ALS (Wittstock et al.). Lastly, few studies report on reduced interhemispheric inhibition (IHI) using transcranial magnetic stimulation (TMS) (Wittstock et al., 2010, Karandreas et al., 2006; Salerno & Georgesco, 1998). It is not known whether there is any correlation between these different findings. Here, we studied the integrity of the CC in ALS on the morphological, the functional-electrophysiological and the clinical level. Methods: We studied 29 right-handed ALS patients (9 bulbar onset) and 21 healthy right-handed controls. MM in the left hand during movements of the right index finger were quantified using surface EMG. Diffusion tensor imaging tractography was used to segment the CC and quantify fractional anisotropy (FA). IHI was studied as a marker of CC function using a double-pulse TMS protocol (Ferbert et al., 1999) at different stimulus intensities in 11 patients and 12 controls. Results: ALS patients showed significantly decreased FA in the motor segment of the CC ($p<0.01$) and IHI was significantly decreased at high stimulus intensities compared to controls ($p=0.016$). Yet, patients did not show increased MM. Conclusion: We showed that the functional integrity of the CC is altered in ALS, using a double-pulse TMS protocol specifically targeting the transcallosal pathway between motor cortices. IHI was significantly reduced in ALS, correlating with decreased FA in the motor CC. Interestingly, patients did not exhibit increased MM compared to controls. IHI might serve as a marker of transcallosal pathway disruption in ALS, even before functional deficits become apparent. Also, ALS patients may be able to compensate for callosal disruption using other functional pathways.

P.40. Euro-MOTOR: A case-control study of hormonal exposures as risk factors for ALS in women

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Introduction: Lifetime exposure to unopposed oestrogen has previously been reported as a protective factor against ALS amongst women. We aimed to replicate these results in a large case-control study. Methods: ALS cases and matched controls were recruited over 4 years in Ireland, Italy and the Netherlands. Hormonal exposures including reproductive history, breastfeeding, contraceptive use, hormonal replacement therapy and gynecological surgical history were surveyed in participating women. Lifetime exposure to unopposed oestrogen was calculated from survey data. Logistic regression/ conditional logistic regression was used to calculate odds ratios. Results: 652 patients and 1217 controls were included. 270 patients (41%) had bulbar onset disease. Lifetime unopposed oestrogen exposure (calculated by subtracting the duration of pregnancies and of oral contraceptive use, and the number of post-ovulatory weeks from the reproductive time-span) was lower in the Netherlands (median 14.6 yrs) vs Ireland (median 16.4 yrs) and Italy (median 18.2 yrs). 30% of Italian, 42% of Irish and 73% of Dutch women had a history of oral contraceptive use. Unopposed lifetime oestrogen exposure did not differ between case and controls within countries or after multivariate adjustment for country, age and education. However duration of oral contraceptive use was higher in controls (median 14 yrs) versus cases (median 8 yrs). The odds ratio for oral contraceptive after correction for age, country, and education was 0.90 (0.84 - 0.97) for 5 years of use. The direction of the relationship was consistent when stratified by country, although not significant in the Netherlands. Discussion: These results are at variance with previous findings, which may in part be explained by differential rates of oral contraceptive use across different countries. Nevertheless hormonal factors appear to modify risk of ALS. The precise nature of the relationship remains unclear.

P.41. Deep phenotyping of Frontotemporal Dementia (FTD) and FTD-MND in Ireland

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Deep phenotyping of Frontotemporal Dementia (FTD) and FTD-MND in Ireland (A clinic-based cohort longitudinal study): Background: Frontotemporal Dementia (FTD) encompassing behavioural and language variants, is genetic in up to 50% of cases. Objectives: 1- to perform deep phenotyping of Irish patients with FTD. 2- to explore familial aggregation for other neuropsychiatric disorders in relatives. Methods: 53 clinic based prevalent patients are enrolled in a longitudinal case control cohort study including detailed neuropsychology and family history evaluation, 3 monthly Motor Unit Number Index (MUNIX) testing, 4 monthly spectral EEG and 3T MRI over 3 years. Results: Of 53 enrolled patients (M: F ration: 26:27) 18 with behavioural variant FTD, 11 with Progressive Non-fluent Aphasia (PNFA), 2 with Semantic Dementia (SD), 14 with FTD-MND (Motor Neurone Disease), 4 with Progressive Supranuclear Palsy (PSP), 3 with Corticobasal syndrome (CBS) and 1 with PNFA-MND. The mean age at onset is 64.4 years (range 53 to 76 years). The time interval between symptom onset to diagnosis ranged between 4 months and 6 years (mean 25 months). 18% of patients have evidence of an autosomal dominant inheritance. Higher rates of other neurodegenerative disease including MND, Parkinson Disease (PD) & Alzheimer Disease (AD): (57%); neuropsychiatric disorders including Schizophrenia, Mania, Bipolar affective disorder and Depression: (36%); Learning disability (20%) and Suicide (12%) are present in first and second-degree relatives (N=1012). Lower MUNIX values are present in FTD-MND and bv-FTD patients suggesting early involvement of anterior horn cells in some instances. Conclusion: Deep phenotyping of FTD provides a rich data source for biomarker profiling and genotype-phenotype correlations Keywords: FTD, ALS, Phenotyping.

P.42. Critical issues in the Euro-MOTOR case-control study

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Despite a large number of case control studies performed in ALS, no environmental factors have been definitely established. Euro-MOTOR is an international multi-institutional case-control study which represents a great opportunity to examine

several risk factors at the same time in different geographic areas. Incident ALS patients (1,631; 36%) and matched controls (2,922; 64%) were recruited in The Netherlands, Italy and Ireland, with a 1:1.8 case-control ratio. Patients reported a median age of 66 years (IQR=58.5-72.2) and were 58.6% male. Half of patients were enrolled in The Netherlands (51%), 38% in Italy and 11% in Ireland. The site of ALS onset was mainly spinal (64%), although it was significantly more common in Ireland (75%) than in Italy (66%) and in The Netherlands (61%). A total of 1,257 (77%) cases underwent a genetic analysis to identify the GGGGCC repeat expansion in the first intron of the gene C9orf72. Cases and controls were matched by gender, age (Â± 5 years) and location (general practitioners' practice or local area). Both individual and frequency matching were used. The population of the study sample has been guaranteed by the active ascertainment of consecutive patients with ALS living in the study areas, where population registries are active. Several sets of indicators were operationalized on the basis of theoretical hypotheses and findings from the literature: ancestry and residential history; life-style habits (smoking, alcohol consumption, physical activities and hobbies); reproductive history; medical history and use of drugs; family medical history; occupations; trauma and injuries. The purpose of this contribution is to describe the study design and methods, highlighting strengths and limitations. Although special attention was paid to methodological aspects, the different socio-cultural systems in the three countries played an important role on defining and interpreting results and clinical characteristics of the disease.

P.43. Coexistence of ALS and CADASIL in an Albanian patient

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Amyotrophic Lateral Sclerosis (ALS) and Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarct and Leukoencephalopathy (CADASIL) are two rare diseases. Here we report a rare case of association of the two diseases in a 48-year-old Albanian man. The patient came to our attention for progressive weakness and muscle wasting at the four limbs, with onset two years earlier. He never complained of migraine. His family history revealed that is mother, dead at the age of 75, presented an ischemic stroke at 69 and, since then, concomitant cognitive impairment. The neurological examination showed fasciculations, muscle wasting, weakness and increased deep tendon reflexes at the four limbs. Plantar responses were both extensor. The bulbar district was spared. It wasn't possible to perform a cognitive assessment because of language barrier (the patient spoke only Albanian); anyway, no significant cognitive impairment was present, as evaluable. Electromyography revealed chronic and active denervation in the four limbs. Motor evoked potentials from the four limbs showed motor conduction failure. Brain MRI revealed diffuse symmetric bilateral hyperintense

lesions in the periventricular white matter of the frontal, temporal and parietal areas in the FLAIR images. CSF analysis was normal. Left biceps brachii biopsy confirmed denervation atrophy. In view of MRI abnormalities, CADASIL was suspected. NOTCH3 gene analysis unveiled a heterozygous mutation in the exon 4 (p.Arg133Cys). A diagnosis of concomitant ALS and CADASIL was formulated. There is increasing evidence that extramotor areas can be affected in ALS, causing sensory, cognitive and extrapyramidal involvement. CADASIL in association with ALS have also been reported in two cases (Hee-Jin, 2012; Praline, 2010). Mutations in NOTCH3 gene cause cerebral and systemic arteriopathy with consequent reduction of cerebral blood flow, as previously found. Therefore, it could be possible that mutations in NOTCH3 contribute to ALS pathogenesis through hypoxic injury and oxidative stress, promoting motor neuron apoptosis.

P.44. Clinical epidemiology of amyotrophic lateral sclerosis in liguria, Italy

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Objectives: To assess Amyotrophic Lateral Sclerosis (ALS) incidence and its trend over time in Liguria, an Italian north-western region, performing an analysis of data prospectively collected from January 1, 2009, to December 31, 2014. To determine the mean and median survival in the 2009-2014 Ligurian ALS incident cases and to evaluate the presence of disease prognostic factors. Methods: The Liguria Register

for ALS (LIGALS) is an ongoing, multicentre prospective register enrolling all ALS incident cases in Liguria. Cases were identified using several concurrent sources. ALS diagnosis is based on El Escorial revised criteria (EEC-R). Results: During the six-year surveillance period, 298 patients were enrolled in this study. The mean annual crude incidence rate in the 2009-2014 period was 3.11/100,000 population (95% CI, 2.77 to 3.49). Survival analysis demonstrated a median survival from symptom onset of 37.0 months (CI 95% 32.0-42.0). Conclusions: ALS crude incidence in Liguria is higher when compared to data from studies conducted in other Italian regions with a similar design. Clinical and epidemiological data are comparable with those of the Italian ALS population. Survival analysis showed that age at onset, site of onset, EEC-R diagnostic category and diagnostic delay are related with survival outcomes.

P.45. CASE 1

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A 70 year old man with history of moderate enolism and obstructive pulmonary disease treated with long course of corticoids was referred with head drop and progressive trunk weakness of two months of duration. During the next three months the weakness became more severe affecting extremities with predominantly proximal involvement. Concomitantly increasing respiratory distress, dysarthria and dysphagia appeared. First physical examination revealed severe trunk and asymmetric proximal weakness. Tendon reflexes were 2+/4 throughout, except both aquileus reflexes were absent. Sensory testing showed only a minimal reduction in vibratory sensation at the toes. Nerve conduction studies showed diffuse axonal loss with no motor conduction block. Nerve conduction velocities were normal. Sensory nerves were mildly affected with distal amplitude of snap decreased in both sural nerves. There were a mild neurogenic recruitment pattern in both legs and denervation activity in thoracic and lumbar paraspinous muscles. Serum IgM anti-GM1 and antineural antibodies were negative. All serology and toxics analysis were normal. Spinal fluid analysis was also normal. IRM cranial and holomedular and were normal. According to the severe progressive disease patient was treated with five cicles of intravenous immunoglobulins with no results. Second electromyography already showed diffuse denervation. Central Motor conduction time was study by motor potential evoked by transcranial magnetic stimulation (TMS) and it showed an increased latency affecting cervical medullar in both arms. The latters gave evidence of lower and upper motoneuron involvement according to ALS disease. In the present moment he is prostrated with palliative treatment requiring assisted ventilation with bipap with no nutritional enteral support according to patient decision. Comment This patient had relatively rapidly progressive weakness with head drop and trunk involvement as a first symptom of disease, progressive evidence of respiratory and bulbar involvement of lower, but not upper, motor neurons. There was no evidence of demyelination or serum antibodies to suggest an immune etiology for the syndrome. Although denervation in the thoracic paraspinous muscles is more suggestive of a motor neuron disease than a motor neuropathy, this clinical course is more rapid than that usually

seen in MMN. At the first time of disease there were not enough evidence for ALS disease. This is a case of a proximal lower motor neuron syndrome with diffuse motor neuropathy. Because of rapidly progression we though that immunosuppressive was an alternative treatment although he did not respond. Focus on electrodiagnostic studies, the central motor study with TMS is an objective profiling tool to evaluate upper motor neuron involvement, been highly recommended to provide diagnostic criteria for ALS disease.

P.46. Brain computer interface with P300-Speller: Feasibility of use for disabled ALS patients

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Introduction: Ability to communicate is a main concern in supportive care of ALS patients. BCI with P300-speller can restore communication. The usefulness of BCI communication devices has been reported in ALS and needs to be improved. Purpose: To demonstrate the feasibility of use of P300 speller and to evaluate efficiency and usability in the context of severe disabled ALS patients. Methods: Each patient participated in 2 identical sessions including (i) a copy-spelling task of two imposed 10-letter words, (ii) a free-spelling task in typing four 5-letter words and (iii) a free-used optional task to type sentences of his/her choice. VAS scores (on a scale of 10) about usability, operability and motivation to use the device were also assessed. Results: 20 ALS patients followed in the ALS Centre of the University Hospital of Nice (H/F=10/10; mean age=62; mean ALSFRS-R score=26/48; mean Norris bulbar score=17/39) were included. The P300 speller achieved a feasibility score of 100 % as all patients succeeded in copy and free-spelling tasks for both sessions. Median efficiency was 95% for copy-spelling and 92,5% for free-spelling. The system accessibility was possible for all patients whatever the degree of disease or age and patients' satisfaction (average score) was for comfort, ease of use and usefulness of 8.1/10, 8.4/10 and 9.75/10, respectively. Conclusion: Our study confirms the feasibility of use of P300 speller for severe disabled ALS patients, with really good performances. At the end of the sessions, all patients used the system for free-use without asthenia or attention difficulties and satisfaction was high in all evaluated domains. In order to respond to daily situations, several technical improvements have to be developed. Acknowledgment: ARSLA for financial support.

P.47. BMAALS: is there a link between ALS and BMAA exposure?

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Objective: To measure the spatial interactions between ALS incidence and the presence of cyanobacteria producing BMAA within the local environment of ALS patients. Methods: ALS cases were identified during 2003-2011, by using multiple sources (ALS national database, insurance health and hospital medical data) from 10 French counties of Limousin, Languedoc Roussillon and Rhone Alpes. Spatial analysis defined areas with an overincidence based on standardized incidence ratio. Kulldorff and Moran tests allowed to distinguish clusters from probable and possible areas of overincidence. Individual and collective samples (n=155, water and food) were assessed for dosage of BMAA by using LC-MS/MS method which has been validated for the detection of underivatised L-BMAA at trace levels in complex environmental matrices. Four samples of 10 ALS patients' brain (hippocampus, frontal lobe, primary motor cortex and mamillary tubercle) were analyzed. Otherwise, a microbiological approach studied the production of BMAA by different strains of cyanobacteria. Results: The exhaustiveness of the incident cases varied from 80% in Rhone Alpes to 99% in Limousin. 1199 ALS patients were identified (sex ratio: 1.2, FALS: 3.7%, spinal forms: 67%, bulbar forms: 31%) corresponding to a standardized incidence of 2.46/100 000 persons-years of follow-up. The geoepidemiological approach highlighted one cluster with 50 ALS cases, 6 areas of probable overincidence and one possible area of overincidence. Trace levels of BMAA was only found in one sample of mushroom. L-BMAA was not detected in brains of ALS patients. The 2,4-DAB was present in some environmental and brain samples. Microbiological analysis found traces of free and bound BMAA and significant levels of 2,4-DAB in some strains of cyanobacteria. Discussion: This study does not confirm the hypothesis of a possible interaction between a BMAA exposure and the risk of ALS. An international inter-laboratory test is planned to compare the results of BMAA dosage in brain.

P.48. Big data, collaborations and patient centricity in ALS

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OBJECTIVE: Implement collaborative patient-centered environment, methodology, processes and distributed infrastructure for creating "Big Data" in ALS. BACKGROUND: Large datasets are critical for identifying statistically significant and biologically relevant observations. Heterogeneity of ALS complicates disease modeling and potential therapies development, making clinical trials challenging. Proposed platform for patient-centered clinical and research information aggregation, collaboration and sharing plus properly aligned incentives allow ALS to enter the Age of "Big Data". DESIGN/METHODS: Patient-centered approach requires unique patient identification across clinical research continuum. Global Unique Identifiers (GUIDs) allow linking phenotypical data to biospecimens, patient-reported

outcomes, genetic files and images across clinical and research encounters regardless where/when information was collected, thus giving researchers access to patient information collected elsewhere. Stakeholders include clinicians, research and commercial biobanks, academic consortia, patient organizations, foundations, industry and patients. Collaboration models for information sharing are essential. RESULTS: Electronic Health Records and research data, distributed "virtual" biobanks, image banks and genetic files are linked by GUIDs. NeuroBANK, a patient-centered accelerated clinical research platform, is in the center of this clinical research universe. Harmonized PRO-ACT dataset provides insights into PALS population participating in clinical trials. Similar dataset from observational studies and medical records will revolutionize our knowledge of ALS natural progression. Standard procedures, common consent language and technology unify multiple participants of clinical care and research (physicians, researchers, industry, patients and non-profits) and informational components (clinical data captured at bedside and from health records, research data, patient-reported outcomes, and data from completed clinical trials) into single disease-specific research continuum. Several disease networks, such as ALDConnect and NEALS ALS consortia, successfully implemented this approach. CONCLUSIONS: Extremely powerful concept of Patient Centricity in clinical research paired with modern technology like NeuroBANK, and PRO-ACT and incentives to collaborate create clinical research continuum from bedside to clinical trials and back to research and clinical care.

P.49. Artificial Neural Networks in Forecasting ALS progression from Clinical Data

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We present the results of a research project aimed at using artificial intelligent and data mining methods to forecast disease progression in ALS. In particular, we present results concerning data visualization, clustering and artificial neural networks obtained by studying the ProACT database. The first part of the work concerns the analysis of the dataset to check if available data mirrors the behavior of the disease as it would be expected from a clinical viewpoint. This analysis has allowed us to identify the exogenous variables for training an artificial neural network for forecasting the behavior of clinical data. In particular, forecasts for FVC, FRS and BMI at time t (endogenous variables), have been obtained training the network on the exogenous variables Gender, Onset (bulbar, limbs), age at onset, time to diagnosis, as well as the rate of decline of FRS, FVC and BMI between symptom onset and the first evaluation. The ANN structure (number of layers and neurons) has been optimized using a subset of 450 patients for which full longitudinal were available. Training, validation and test show that the trained ANN predictions come with very low root mean square error. Results are promising for building a decision aid tool that can help clinicians in forecasting the evaluation of the disease in anticipation, which is especially relevant when delicate decisions concerning medical interventions (e.g. PEG, NIV) are to be taken.

P.50. ALS outcome measures and the role of smoke and vascular risk factors: a population-based study

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Objective. To assess the prognostic influence of pre-morbid smoking habits, diabetes, hypertension and vascular risk profile on ALS phenotype and outcome in a population-based cohort of Italian patients. Methods. A total of 650 ALS patients from the Piemonte/Valle d'Aosta Register for ALS, incident in the 2007-2011 period, were recruited. Information about premorbid smoke habits, diabetes mellitus, arterial hypertension were collected at the time of diagnosis. A vascular risk profile of patients was calculated according to the Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice (JBS2). Results. Current smokers had a significantly shorter median survival (1.9 years, interquartile range [IQR], 1.2-3.4) compared to former (2.3, IQR 1.5-4.2) and never smokers (2.7 years, IQR 1.8-4.6) (p=0.001). Diabetes and arterial hypertension did not influence ALS prognosis. Patients with a lower vascular risk profile had a better prognosis than those with intermediate and higher risk profiles. Conclusions. This study has demonstrated in a large population-based cohort of ALS patients that smoking is an independent prognostic factor, with a dose-response gradient. The understanding of the mechanisms, either genetic or epigenetic, through which exogenous factors influence disease phenotype is of major importance toward a precision medicine approach for the cure of ALS.

P.51. ALS Centre Moscow: 4 years' experience

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We started multidisciplinary ALS centre Moscow in 2011. We provided care for 663 patients (317 males and 346 females) in Moscow and other Russian cities. Mean age of ALS onset in our patients is 59,3 Â±10,8 years with median disease duration of 1188,0 (791-1787) days. Median time from disease onset to diagnosis was 365 (191-587) days, median time of referral to our centre was 320 days and median time of follow up was 387 days. NIV prolonged median survival by 66 days.

P.52. Acoustic reflex patterns in amyotrophic lateral sclerosis

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Objective: To investigate acoustic reflex testing in ALS patients. Design: Amplitude, latency and rise time of stapedial reflex were recorded for 500 and 1000 Hz contralateral stimulus. Statistical analysis was performed by Wilcoxon test and the level of significance was set at 5%. Study sample: 51 ALS patients and 10 sex- and age-matched control subjects were studied. Patients were further divided in two groups: ALS-B (38 cases, with bulbar signs at evaluation) and ALS-S (13 cases, without bulbar signs at evaluation). Results: Stapedial reflex was present in all patients. There was a statistically significant difference in the mean amplitude, latency and rise time between the ALS patients as compared with the controls. Amplitude was lower in both the ALS-B and the ALS-S patients than in the controls ($p<0.05$) and rise time was longer in both patient groups compared with the controls ($p<0.05$). Conclusions: These results confirm the presence of abnormal acoustic reflex patterns in ALS cases with bulbar signs and, moreover, suggesting a possible subclinical involvement of the stapedial motor neuron even in ALS-S patients. Amplitude and rise time seem to be good sensitive parameters for investigating subclinical bulbar involvement.

P.53. A population-based case-control study to assess sleep disturbances as a risk factor for ALS

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Introduction ALS is thought to be a multistep process in which (epi)genetic and environmental factors are involved, and defective removal of toxic proteins is considered one of the pathogenic factors. From mice studies, it has been suggested that sleep has a critical function in ensuring metabolic homeostasis in the brain by enhanced clearance of neurotoxic waste products. Sleep disturbances are common in ALS patients. However, it is unknown whether sleep disturbances are a risk factor for ALS. Therefore, the objective of this population-based case-control study was to assess the risk of developing ALS in relationship to presymptomatic sleep quality. Methods We recruited 261 ALS patients (response rate (RR) = 74%) and 539 age- and gender matched controls (RR=70%) through the Prospective ALS study the Netherlands. Subjects filled out a questionnaire assessing sleep quality, nocturnal hypoventilation and presence of sleep disorders 1, 5 and 10 years before onset or inclusion. To assess the association between sleep quality and ALS, multivariate models (covariates BMI, education, smoking and alcohol) were used. Questionnaire outcomes were clustered using principal component analysis (PCA). Component

scores were compared between cases and controls. The association between sleep quality and ALS risk over time was studied using linear mixed models. Results Nocturnal restless legs syndrome (RLS) symptoms, muscle cramps and leg twitching were more prevalent one year before onset of muscle weaknessÂin patients (adjOR 1.67 (1.09-2.54), 2.75 (1.88-4.02) and 3.35 (2.12-5.32) respectively). Time spent in bed was similar for patients and controls (p-values>0.05). Four PCA clusters were identified; the cluster of leg complaints showed that this is more common one year pre-onset compared to controls ($p<0.0005$). Conclusion Nocturnal leg complaints, such as RLS, cramps and twitching, should be regarded as prodromal symptoms. Further analyses on presymptomatic sleep quality as risk factor for ALS will be presented at the meeting.

P.54. A case of late-onset Obsessive Compulsive Disorder developing Upper MND and Frontotemporal Dementia

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We describe a 64 year-old woman, who developed an Upper Motor Neuron Disease (MND) which occurred 5 years after the appearance of a late-onset Obsessive Compulsive Disorder (OCD). At age 57, the patient began to complain of anxiety, ego-dystonic obsessions and repetitive behaviors. An OCD was diagnosed and she underwent an antipsychotic treatment without benefit. Familial and remote histories were negative for neuropsychiatric disease. The disturbances were severe enough to negatively impact on her quality of life. Five years later, she presented to our MND Centre with muscle weakness in her left leg, dysarthria and dysphagia. Neurological examination showed paretic-spastic gait, pyramidal signs (Babinski sign, diffuse hyperreflexia) and mild weakness of proximal muscles Electromyographyshowed neither fasciculations nor active denervation. Neoplastic and infection markers, onconeural antibodies and cerebrospinal fluid (Tau, P-Tau, ABeta-42 andisoelectrofocusing) were normal. Neuropsychological evaluation showed an intact cognitive functioning. We diagnosed an Upper MND without mutations in FUS, TARDBP, GNR and C9orf72 genes. Brain Magnetic Resonance (MRI) showed a bilateral hippocampal atrophy with cortical sclerosis of the right hippocampus andPositron Emission Tomography (PET) disclosed a moderate right temporal cortex hypo-metabolism. Six months later, both motor neuron and psychiatric symptomsdeteriorated. A neuropsychological assessment revealed a long-term memory deficit and executive dysfunction. MRI and PET showed a severe worsening of atrophy andhypo-metabolism in frontotemporal lobes. We believe our patient suffered from a MND and Frontotemporal Dementia (FTD). To our knowledge, this is the first reported case of MND/FTD whose initial presentation consisted of a late-onset ODC.

P.55. Perception of dignity in ALS patients and its influence on emotional distress

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Introduction ALS patients suffer intense emotional distress. The dignity model of Chochinov is a therapeutic framework that can guide patients, families and professionals to better identify their essential needs and intervene in them reducing their emotional distress, but so far, has been used primarily with oncology population. There is a screening test (CED-PAL) validated to analyze dignity in non-cancer patients in Spanish population. The degree of spirituality and couple affection can influence patients' dignity and secondarily their emotional distress. Objective To describe the perception of dignity of ALS patients and its influence on emotional distress and quality of life. Analyze the influence of spirituality and relationship satisfaction on the perceived dignity. Methodology Outpatients followed in the ALS Unit were enrolled in this prospective study. Dignity, spirituality, relationship affection, emotional distress and quality of life were assessed with the appropriate scales. Correlations between variables were analyzed. Results Twenty patients were enrolled, all with a long-term relationship being in most cases the couple the primary caregiver. We found a positive correlation between a satisfying relationship and social support and the sense of dignity. A satisfying relationship, a high spirituality and a high dignity correlated with less emotional distress. Dignity correlated positively with quality of life. Conclusions Measures aimed at promoting dignity can positively influence the quality of life and reduce emotional distress. A satisfying relationship may increase patients' perceived dignity.

P.56. Insights into Amyotrophic Lateral Sclerosis from a Machine Learning Perspective

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We apply machine learning (ML) algorithms to the Pooled Resources Open-Access ALS Clinical Trial (PRO-ACT) database and view the value of the ALS patient functional rating scale (ALSFRS) as a descriptor of the disease state. We are interested in the following questions: 1) Can we find physiological and laboratory test variables that are important with respect to the disease state?; 2) Can we build models using these variables that accurately predict a patient's future disease state?; and 3) Can we create models that help to understand and explain the influence these variables and their connections have on the disease state? To address these questions, we train ML algorithms to build models of the disease using data from the first and last clinic visits, visits that were up to two years apart, representing the beginning and end of disease progression, respectively. The models predict the ALSFRS value observed during the last visit using data collected at different disease stages (onset, advanced, or both). Model performance shows that by using data we can explain and predict the state of disease informatively and accurately. Analysis of the learned models reveals a number of interesting patterns that highlight underlying mechanisms of ALS. For instance, we show that the ten ALSFRS values can be statistically clustered into four medically interpretable functional groups, and that certain variables are strongly related to specific groups, while less so to others (e.g., chloride is a strong predictor for bulbar functions, whereas creatinine is connected with large muscle functions). Furthermore, we introduce explanatory models and demonstrate their potential to highlight complex relationships among variables. We then leverage these relationships to identify combinations of variable values that differ between mild and severe patients. We believe the suggested methodology and identified patterns and relationships will advance efforts to understand ALS.

Cognitive/Behaviour

P.57. The validation of the Italian Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

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Objective: The study presents the Italian validation of the recently developed Edinburgh Cognitive and Behavioural ALS Screen (ECAS), a short screen for cognitive/behavioural alterations in patients with amyotrophic lateral sclerosis (ALS). We evaluated the psychometric properties of the ECAS Italian version in terms of reliability and convergent validity for both cognitive and behavioural features. Furthermore, we investigated the relationship with affective and clinical variables, in addition to ECAS usability and patients' insight into cognitive/behaviour changes. Finally, correlations between genetic and cognitive/behavioural data were analysed. Methods: We recruited 107 ALS patients. Normative data were collected on 248 healthy subjects. Participants were administered the ECAS and two standard cognitive

screening tools (FAB; MoCA), two psychological questionnaires (BDI; STAI/Y) and an ad-hoc usability questionnaire. The FBI was also carried out with caregivers. Results: With respect to the original English version, some adaptations to Italian language and culture were applied. As cognitive performance was found to be significantly related to age and educational level, age-education adjusted cut-offs for the Italian population were provided. The ECAS Italian version discriminated well between patients and controls. The most prevalent deficit occurred in executive functions and fluency. Correlations were observed between the ECAS and standard cognitive screening tools and between the ECAS carer interview and the FBI, supporting its full convergent validity. A low prevalence of cognitive-behavioural alteration was found in our genetic mutation carriers. Conclusions: The ECAS Italian version provides clinicians with a rapid, feasible and sensitive tool, useful to identify different profiles of cognitive-behavioural impairment in ALS. Moreover, since the ECAS provides a dual mode of execution, it better fits to ALS clinical spectrum with respect to other widely used traditional screening tools and it can be used also in moderate stages of the disease.

P.58. The relationship between Apathy and Executive Dysfunction in ALS

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Background: Behaviour change in ALS is primarily characterised by apathy. We have previously shown that apathy is multidimensional and the apathy subtype found in ALS is Initiation apathy (self generation of thoughts). However the relationship between this apathy subtype and the cognitive profile, in particular executive dysfunction, in ALS is unknown. It is our hypothesis that specific cognitive deficits underlie this particularly prominent behavioural symptom with most likely a common underlying cause. This study aimed to examine the relationships between types of executive dysfunction and dimensions of apathy in ALS. Method: 30 ALS patients (and carers) and 34 healthy gender-age-education matched controls (and informants) were recruited. All completed the Geriatric Depression Scale, Apathy Evaluation Scale and Dimensional Apathy Scale (DAS). The DAS was used to classify apathy subtypes. Patients and controls completed the ECAS, a neuropsychological battery containing Planning and Goal management tasks, emotional perception (Ekman 60 Faces), social cognition (Judgement of Preference) and intrinsic response generation (Verbal Fluency and Random Number Generation). Results: Initiation apathy was the most commonly observed apathy subtype in ALS patients. Patients scored significantly higher than controls only on Initiation apathy (self: U=246, p=.0004; informant/carer: U=255, p=.0006), with no differences on Executive or Emotional apathy. Patients showed a significant deficits compared to controls in verbal fluency (U=253, p=.0006), Ekman 60 Faces test (U=349.5, p=.03), Judgement of Preference task (U=387.5, p=.006), shorter planning task time (U=318, p=.01), impaired goal time management (U=293.5, p=.004) and difficulty randomizing numbers (U=356, p=.04). Patient correlational analyses showed significant associations between the verbal fluency deficit and Initiation apathy (self: r(28)=-.51, p<.01 carer: r(28)=-.45, p<.05) and not with depression. No such associations were found in controls. Conclusion: Verbal fluency deficits commonly reported in ALS patients are associated with increased Initiation Apathy, indicating a possible common underlying cause to both of these symptoms.

P.59. Standardization and Validation of the ECAS using Age and Education Adjusted Norms

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Background: The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is a brief multi-domain cognitive test developed for the screening of cognition and behaviour in ALS. The ECAS was developed to meet the need for a short clinical screening tool sensitive to the varying prevalence and heterogeneity of cognitive impairment in ALS. Objective: The aim of this study was to validate the ECAS against a standardized neuropsychological battery using age and education adjusted cut-off scores. Method: 30 incident ALS cases underwent both ECAS and extensive neuropsychological assessment. A sample of 82 healthy controls was assessed on the ECAS to create age and education adjusted cut-off scores. Results: High correlations were observed between ECAS composite scores (ECAS Total, ALS Specific and ALS Non-Specific) and full battery composite scores. ECAS cognitive domains and subtests also correlated highly with their analogous from the gold standard neuropsychological battery. The ECAS Total, ALS Specific and ALS Non-Specific scores were found to be highly sensitive to cognitive impairment in ALS. ECAS ALS-Specific cognitive domains (Language, Verbal Fluency and Executive Function) also showed high sensitivity. Individual subtest sensitivity was medium to low, suggesting that their use should be interpreted with caution. Low positive predictive values indicated the presence of false positives. Conclusions: Findings of this study indicate that the ECAS is a valid measure to screen for cognitive impairment in ALS using age and education adjusted norms when used as an overall measure of cognitive decline. Further comprehensive cognitive assessment is required for patients that present as impaired on the ECAS.

P.60. Slovenian version of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) - preliminary results

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Specific cognitive changes, including deficits in executive functions and changes in language and social cognition, are commonly found among patients with ALS. Cognitive dysfunction can impair patient's decision making related to disease management. 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 non-specific cognitive functions and a carer behaviour screen. In order to use the ECAS in Slovenian speaking patients with ALS, we translated and adapted the original version of ECAS. To obtain normative values and cut-off scores, 20 healthy controls (mean age 68, range 52-79 years) were tested using the Slovenian version. ECAS was used in 20 patients with ALS (mean age 68, 51-87 years). The cut-off scores, calculated from the control group, were comparable to those published for the Swiss-German version of ECAS [2] but lower that those in the original English version [1]. In our patient group, the percentage of patients below the cut-off for the ALS-specific score was slightly higher (35%) than in the English (29%) and in the Swiss-German version (21%). The difference was larger for the ALS non-specific score and total score. Higher percentage of cognitively impaired patients with ALS found using the Slovenian version of ECAS compared to the English and Swiss-German versions might be the consequence of older age of the subjects in our group. ECAS is a useful tool to identify these patients and can improve the quality of care offered by the multidisciplinary ALS team. 1. Abrahams S et al. Amyotroph Lateral Scler Frontotemporal Degener 2014; 15: 9-14. 2. Lulé D et al. Amyotroph Lateral Scler Frontotemporal Degener 2015; 16: 16-23.

P.61. Psychophysiological principles underlying pathological laughing and crying in ALS

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Background: The syndrome of pathological laughing and crying (PLC) is characterized by episodes of involuntary outbursts of emotional expression. Although this phenomenon has been referred to for over a century, a clear-cut clinical definition is still lacking, and underlying pathophysiological mechanisms are not well understood. In particular, it remains ill-defined which kind of stimuli - contextually appropriate or inappropriate - elicit episodes of PLC, and if the phenomenon is a result of a lack of inhibition from the frontal cortex ("top-down-theory") or due to an altered processing of sensory inputs at the brainstem level ("bottom-up-theory"). Methods: We studied ten ALS patients with PLC and ten controls matched for age, sex and education. Subjects were simultaneously exposed to either emotionally congruent or incongruent visual and auditory stimuli and were asked to rate pictures according to their emotional quality. Changes in physiological parameters (heart rate, galvanic skin response, activity of facial muscles) were recorded, and a standardized self-assessment lability score (CNS-LS) was determined. Results: Patients were influenced in their rating behaviour in a negative direction by mood-incongruent music. Compared to controls, they were influenced by negative stimuli, i.e. they rated neutral pictures more negatively when listening to sad music. Patients rated significantly higher on the CNS-LS. In patients, changes of electromyographic activity of mimic muscles during different emotion-eliciting conditions were explained by frontal cortex dysfunction. Conclusion: We conclude that PLC is associated with altered emotional suggestibility and that it is preferentially elicited by mood-incongruent stimuli. In addition, physiological reactions as well as behavioural changes suggest that this phenomenon is primarily an expression of reduced inhibitory activity of the frontal cortex, since frontal dysfunction could explain changes in physiological parameters in the patient group. We consider these findings being important for the clinical interpretation of emotional reactions of ALS patients.

P.62. Psychological status and emotional burden in ALS caregivers: the role of metacognitive processes

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The high caregiver burden in Amyotrophic Lateral Sclerosis (ALS) relates to personal and social restrictions and to psychological and emotional problems and has been shown to influence patients' psychological and emotional status. Metacognition is defined as "the aspect of information processing that monitors, interprets, evaluates and regulates the contents and processes of its organization". It predicts the development of anxiety and depression symptoms especially in presence of life-stress events. Dysfunctional metacognitions contribute to the activation of an unhelpful, perseverative style of information processing in health-related stressful situations. The aim of this study was to evaluate how the metacognitive processes may influence emotional status, burden and coping strategies in ALS primary caregivers. A total of 70 primary caregivers of ALS patients participated in the study. The Metacognitive beliefs and processes were evaluated by the Metacognitions Questionnaire 30 (MCQ-30). Anxiety and depressive symptoms were assessed with the State-Trait Anxiety Inventory (STAI) and the Beck Depression Inventory II (BDI-II). Caregivers' coping strategies, needs and burden were assessed with the Brief COPE, Caregiver Burden Inventory (CBI), ALS Caregiver Needs and Burden Questionnaire (ALS CNB-Q). Metacognition (MCQ-30 total score) was significantly related to state and trait anxiety ($p < 0.01$), cognitive and somatic aspects of depression ($p<0.01$) and also caregiver burden (CBI and ALS CNB-Q $p<0.01$). Among the different sub-scales, MCQ_NEG subscale "Negative beliefs about worry concerning uncontrollability and danger" showed the strongest correlations with all the above mentioned aspects ($p <0.0001$). Metacognition (MCQ-30 total score) was related with the maladaptive strategy of "coping behavior disengagement" (subscore BRIEF_BD,$p<0.01$). Among the different

sub-scales, MCQ_NEG subscale showed the strongest correlations with maladaptive coping strategies as denial and venting (p <0.0001). Our results showed that metacognition can be considered as a important factor ables to influence the psychological status, emotional burden and coping strategies in ALS caregivers.

P.63. New frontiers of cognitive assessment in neurodegenerative disease: proof of concept by means of ET

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Background and objective: Cognitive assessment in neurodegenerative conditions involving physical disability, such as Amyotrophic Lateral Sclerosis (ALS), is often prevented due to the presence of verbal-motor impairment, especially in moderate-advanced stages of the disease. Even if preliminary attempts have been performed in order to overcome the limitations of traditional screening tools, to date an extensive and fully motor-verbal free neuropsychological battery is not available. We adapted a set of neuropsychological tests for eye-tracking (ET) control, and evaluated their validity and usability features in a sample of healthy participants. Methods: Thirty healthy subjects underwent an ET-based neuropsychological battery, assessing language, attentional abilities, executive functions and social cognition; besides, standard cognitive tools screening for frontal functioning (Frontal Assessment Battery - FAB), global cognitive efficiency (Montreal Cognitive Assessment - MoCA) and working memory abilities (Digit Sequencing Task) were administered. Psychological measures of anxiety (State-Trait Anxiety Inventory-Y - STAI-Y) and depression (Beck Depression Inventory - BDI) were also collected, together with an investigation of ET usability aspects. Results: Assessment of convergent validity of the ET-based neuropsychological battery against standard cognitive tests revealed congruent and significant correlations between the standard screening of working memory abilities (Digit Sequencing Task) and frontal-executive functions assessed with the ET-based tests. Usability aspects concerning the ET assessment were found to be influenced by both working memory abilities and psychological components. Conclusions: The newly developed ET-based neuropsychological battery provides an extensive assessment of cognitive functions, allowing participants to provide responses independently from motor or verbal input channels. Further studies will be aimed at investigating validity and usability components in different clinical populations with motor-verbal impairments, including in particular ALS patients.

P.64. Executive and non-executive cognitive changes and pseudobulbar affect in ALS

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Background: Impaired emotional regulation expressed as pathological laughing and crying and described as pseudobulbar affect (PBA), is common in amyotrophic lateral sclerosis (ALS). To date, only few studies have examined the relationship between PBA and cognitive impairment in non-demented patients with ALS, mostly focused on executive functions but still failed to report consistent findings. Aim: To investigate executive and non-executive cognitive changes in non-demented ALS patients with and without PBA. Methods: We included 51 patients with ALS (26 without/25 with PBA) diagnosed according to the revised El-Escorial criteria and 22 healthy controls (HC). All patients completed the Centre for Neurological Study-Lability Scale (CNSLS) for pathological laughing and crying with a cut-off score of â‰¥13 for PBA. All participants completed cognitive tests of attention/executive functions, memory, constructions and reasoning. Results: ALS subgroups did not differ in disease-related characteristics (severity; duration; spinal/bulbar onset); yet the PBA group included more patients with current bulbar involvement (p=0.035) and showed higher ADI-Depression-Inventory score (p<0.001). On cognitive functions, we found a main group effect on measures of verbal fluency(p=0.003); selective attention/inhibition(p=0.002); cognitive flexibility(p<0.01); working memory(p=0.04); oral response rate(p=0.003); immediate and delayed verbal recall/forgetting rate(p<0.01); bimanual visuospatial dexterity/organization(p=0.007). Bonferroni post-hoc comparisons revealed significantly reduced performance in both ALS subgroups compared to HC (p<0.05) in the previous measures except of working memory and prose memory where only the PBA group had lower scores. Patients with PBA also performed significantly worse compared to patients without PBA on rate of forgetting (p=0.016) and bimanual visuospatial organization (p=0.010). Conclusions: Irrespective of PBA, patients with ALS show executive and non-executive cognitive impairment consistent with a pattern of extra-motor involvement. However, patients with PBA show more bulbar symptoms and impairments regarding working memory and prose memory, verbal forgetting rate and bimanual visuospatial organization which possibly corresponds to a specific neuroanatomical pattern.

P.65. Depression in amyotrophic lateral sclerosis

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Objectives: To examine the relative risk of depression among patients with amyotrophic lateral sclerosis (ALS), both in terms of depression diagnosis and use of antidepressant drugs, before and after diagnosis. Methods: We conducted a nested case-control study including 1752 ALS cases diagnosed from July 2005 to December 2010 and 8760 matched controls based on the Swedish national health and population registers, to assess the associations of depression diagnosis and use of antidepressant drugs with a subsequent risk of ALS. We further followed the ALS cases after diagnosis to estimate the association of an ALS diagnosis with the subsequent risk of depression and use of antidepressant drugs. Results: Before diagnosis ALS patients were at higher risk of receiving a clinical diagnosis of depression compared to controls (odds ratio [OR] 1.7; 95% CI 1.3-2.3) and the highest risk increase was noted during the year before diagnosis (OR 3.5; 95% CI 2.1-5.6). ALS patients also had a highly increased risk of depression within the first year after diagnosis (hazard ratio [HR] 7.9; 95% CI 4.4-14.3). Antidepressant use was more common in ALS patients than in controls, especially during the year before (OR 5.8; 95% CI 4.5-7.5) and the year after (HR 16.1; 95% CI 11.5-22.6) diagnosis. Conclusions: ALS patients are at higher risk of depression diagnosis and use of antidepressant drugs both immediately before and after diagnosis. Keywords: amyotrophic lateral sclerosis, depression, antidepressant drugs, nested case-control study, matched cohort study

P.66. Cognitive impairments may affect general decisional capacity, not personal therapeutic decisions

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Cognitive impairments appear in 48% of the patients with amyotrophic lateral sclerosis (ALS) and behavioural symptoms are present in 9%. An influence of cognition and behavior on patients' medical decisions (e.g. tube feeding or ventilation) is postulated, but no clinical data proves such a relationship. Thus, clinical data analyzing this issue in a large and prospective cohort of ALS patients is necessary. Methods: In total, N=259 ALS patients were examined for decision status, cognition and behaviour in the sense of FTD. All patients filled out standardized questionnaires about (1) their personal medical decisions regarding the use of therapeutic treatments (e.g. ventilation), (2) hypothetical decisions to turn off those treatments in case of physical decline and (3) a general ideation about legalization of life-shortening treatments in Germany. Cognition was assessed with ECAS and CERAD, changes in behavior was reported for N=169 ALS patients by caregivers. Logistic regression analyses and a principal component analysis were used for statistics. Results: Cognitive impairments were present in 55% of the patients and behavioural changes in 15%. Personal decisions on the use of therapeutic treatments (1) were not associated with cognitive or behavioural changes. But, the general ideation about the legalization of life-shortening treatments (3) was significant influenced by cognition and behaviour. Additionally, statistical variance of this relationship was explained by one common factor, whereas variance of other decisions (1+2) underlay other factors. Conclusion:

Decision making in ALS is primarily determined by physical conditions and medical needs. Concluding from the current findings, decision making with respect to personal choices of therapeutic options is not biased by cognitive and behavioural decline despite that ethical reasoning and general complex ideation might be impaired. Thus, surrogate decision making in mild cognitively impaired ALS patients might not necessarily be required.

P.67. Cognitive assessment in ALS by means of P300-Brain Computer Interface: a preliminary study

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Objective: To investigate the use of P300-based Brain Computer Interface (BCI) technology for the administration of motor-verbal free cognitive tests in Amyotrophic Lateral Sclerosis (ALS). Methods: We recruited 15 ALS patients and 15 age- and education-matched healthy subjects. All participants underwent a BCI-based neuropsychological assessment, together with two standard cognitive screening tools (FAB, MoCA), two psychological questionnaires (BDI, STAI-Y) and a usability questionnaire. For patients, clinical and respiratory examinations were also performed, together with a behavioural assessment (FBI). Results: Correlations were observed between standard cognitive and BCI-based neuropsychological assessment, mainly concerning execution times in the ALS group. Moreover, patients provided positive rates concerning the BCI perceived usability and subjective experience. Finally, execution times at the BCI-based neuropsychological assessment were useful to discriminate patients from controls, with patients achieving lower processing speed than controls regarding executive functions. Conclusions: The developed motor-verbal free neuropsychological battery represents an innovative approach, that could provide relevant information for clinical practice and ethical issues. Its use for cognitive evaluation throughout the course of ALS, currently not available by means of standard assessment, must be addressed in further longitudinal validation studies. Further work will be aimed at refining the developed system and enlarging the cognitive spectrum investigated.

P.68. Assessment of cognitive functions in ALS patients using a new eye movement based ECAS version

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Background: About 30% of patients with amyotrophic lateral sclerosis (ALS) exhibit cognitive deficits, most prominently in the domains of language and executive functioning. However, progressive impairments of speech and movement abilities are a major obstacle in the assessment of cognitive functions in patients with late stage ALS. Yet, reliable neuropsychological assessment plays an important role for clinical practice in the domains of compliance, survival and carer burden. We therefore developed an eye movement based version of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) to test for cognitive deficits in clinical routine. Methods: 37 ALS patients and 50 age-, gender- and education-matched controls underwent testing with the paper-pencil and the oculomotor version of the ECAS. The latter was adapted to the use of the EyeTribe System, which uses infrared illumination to detect eye movements and can be easily connected to a conventional notebook. Participants were presented shortened versions of the ECAS-tasks from all of its five domains (memory, visuospatial perception, language, verbal fluency and executive functioning). They gave their answers, which were recorded by an in-house developed software, by looking at letters and/or numbers displayed on the notebook screen for at least 1000ms Results: Results in the eye movement based version of the ECAS correlated significantly with those of the paper-pencil version in the domains of executive functioning ($p<0.001$) and language ($p<0.001$) and in the overall cognitive performance for ALS patients ($p<0.001$). In addition, a correlation between both versions was observed in all domains (all $p<0.05$) and the ECAS total score ($p<0.001$) in the healthy control sample. Discussion: Our results suggest that eye tracking driven neuropsychological tests provide a viable method to assess cognitive impairment in ALS patients unable to speak or write.

P.69. Reliable Change Index (RCI) as metric for significant change in cognitively intact ALS patients

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Introduction Cognitive impairment is a frequent feature of Amyotrophic Lateral Sclerosis (ALS). Impairment in the executive domain occurs in up to 50% of incident patients with ALS, with deficits in language, emotional processing and social cognition also documented. There has been limited reporting of the longitudinal cognitive profile of those who are normal on initial testing, although some studies have suggested that some patients with normal executive function develop later impairments in language. Methods Cognitively intact ALS patients who completed longitudinal assessment were studied. Using the Boston Naming Test (BNT), a Reliable Change Index (RCI) was calculated to assess whether statistically significant change

was occurring between assessments on language for these cognitively intact patients (n=40), using healthy control data for reliability statistics (n=30). Results Considering the standard error (SE=.514), Standard Difference (SDiff=.728), and reliability (rxx=.811) of the BNT, patient data from T2 was compared to their baseline performance. 32% of patients (n=8) exhibited performances that were above the 95% cut-off for significance. One patient's performance significantly increased on repeat testing, with the remaining 64% experiencing non-significant change. Discussion Language profile, as assessed using the Boston Naming Test remains relatively intact and stable over time in ALS patients who have normal executive function. However, in this study, 32% of cognitively intact patients exhibited a change, while remaining within the remit of an intact cognitive profile. This finding suggests the presence of subtle extra-motor involvement in some ALS patients who do not fulfil the formal criteria for cognitive impairment.

Genes and Genomics

P.70. Whole-Blood Global DNA Methylation in ALS and Trinucleotide Repeat Disorders

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Despite the fact that the overall heritability of ALS is estimated to be well below 30%, large international efforts are on their way to decipher the genetic basis of amyotrophic lateral sclerosis. This is mainly because polygenic, oligogenic and epistatic interactions between different variants, along with compound and incomplete inheritance, could still prove to be crucial in understanding the underlying cellular mechanisms in this devastating disease. Nevertheless, it is quite clear that the majority of ALS may in fact occur not due to genetic, but environmental and epigenetic factors. Here, using a high-throughput ELISA-based assay, we report a significant increase in the global levels of 5mC in ALS patients ($p < 0.001$ [$F(1, 214) = 11.993$, $p = 0.000645$]), when compared to age- and sex-matched healthy individuals, controlling for smoking, physical activity and alcohol consumption. These results conclude disputes between two studies which have reported inconsistent findings regarding this matter; our results also show that such abnormalities are not exclusive to ALS. We have found that the global levels of 5mC are also increased in SCA1 ($p < 0.01$ [$F(1, 32) = 8.778$), $p = 0.00571$]) and SCA2 ($p < 0.01$ [$F(1, 56) = 10.784$, $p = 0.001768$]), but not in Huntington's disease, Friedreich's ataxia and myotonic dystrophy. Finally, we utilised BST-PCR for the quantification of C9orf72 promoter methylation in expansion carrier ALS patients and could not observe a correlation with the global levels of 5mC. Further in-depth analyses are required to shed light on the epigenetic basis of ALS.

P.71. Whole exome sequencing reveals novel and known rare FIG4 mutations in a central European ALS cohort

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Since whole exome sequencing (WES) has become an efficient method for identification of novel and previously known disease mutations in coding regions, important progress in understanding the genetic etiology of amyotrophic lateral sclerosis (ALS) has been made. In this study, we performed WES in an ALS family showing an autosomal dominant inheritance pattern with incomplete penetrance, comprising an index patient and his affected paternal great-aunt. By using an overlapping strategy to identify variants shared by the patient and his father, we detected a rare heterozygous frameshift mutation in FIG4 predicted to truncate the FIG4 protein in its active site. FIG4 encodes a phosphoinositide 5-phosphatase with a key role in vesicle trafficking in eukaryotic cells. Bi-allelic FIG4 mutations have previously been described to cause Charcot-Marie-Tooth disease, type 4J (CMT4J) and Yunis-Varon syndrome. Recent studies showed an association between heterozygous FIG4 mutations and ALS. Therefore, we performed WES or targeted sequencing of FIG4 in 200 ALS patients of mainly central European origin revealing five additional known or novel rare heterozygous missense mutations predicted to be deleterious. Disease duration was relatively long in four of six FIG4 mutation carriers, and three of six displayed a predominant upper motor neuron phenotype. Here, we confirm FIG4 as an ALS risk gene by identification of rare pathogenic variants in 3% of patients in a central European cohort.

P.72. Transcriptome of motor neurons axons derived from pluripotent cells and human spinal motor neurons

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Spinal motor neurons control the initiation of body movement and are severely affected in amyotrophic lateral sclerosis (ALS). These motor neurons have remarkably long axons that connect their somas with target muscles in the periphery through specialized synapses termed neuromuscular junctions. In ALS, motor axons show pathology early in disease with denervation of muscle endplates occurring long before the onset of central motor neuron loss. Consequently, axonal transport is perturbed early in disease, likely affecting local RNA distribution and processing. To investigate the RNA composition of motor neuron somas and axons in health and ALS we have combined microfluidics with next generation sequencing. Here, spinal motor neurons derived from mouse embryonic stem cells (mESCs) were cultured in microfluidic devices. We performed deep-RNA sequencing (RNAseq) on the somatodendritic and axonal compartments of control motor neurons as well as motor

neurons overexpressing wild-type SOD1 or mutant SOD1G93A. To deepen our insight on the transcriptional signatures of motor neurons in ALS we cross-compared RNAseq data from our in vitro system, which represents developing neurons, with adult motor neurons. These adult spinal motor neurons were isolated from human post-mortem tissues using laser capture microdissection and subjected to RNAseq (LCM-seq). In summary, we believe that our study will give new insights on motor axon- and soma-specific disease mechanisms in ALS.

P.73. Transcriptome analysis in a motor neuron muscle microfluidics system in ALS

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Spinal motor neurons are highly polarized cells. Their cell bodies and associated dendrites are located in the spinal cord, while their axons traverse large distances in the body and connect to muscle via specialized synapses termed neuromuscular junctions (NMJs). These NMJs are the first target in the lethal motor neuron disease amyotrophic lateral sclerosis (ALS), where motor neurons disconnect from muscle before their somas in the spinal cord are lost. We created a time course of NMJ pathology in the SOD1G93A mouse model of ALS compared to control littermates. To investigate the neuromuscular connection in ALS and health more in depth, we generated a mouse co-culture system recapitulating the NMJ in vitro. Mouse embryonic stem cell-derived motor neurons were cultured in microfluidic devices together with satellite cells derived from early postnatal mice, which were then differentiated into myofibers. Axons traversed to the myofiber compartment, while motor neuron somas remained separated from the muscle. In this way the only connection between the cell types is at the distal, synaptic end of the motor neuron at the NMJ, mimicking the in vivo situation. Deep RNA sequencing was performed on both the muscle and somatodendritic compartments to investigate local RNA composition. In summary, we reveal the differential RNA composition between motor neuron somas, axons and muscle in health and ALS.

P.74. Role of multiple mutations in disease-causing genes in Italian ALS patients

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ALS-associated mutations have been identified in ~25 genes, often displaying incomplete penetrance and high phenotypic variability, and this finding could in part be explained by the concurrence of multiple rare variants in the same patients. To test this oligogenic hypothesis, we studied the frequency of double mutations in a cohort of 681 Italian ALS patients (180 FALS and 501 SALS). We identified variants in ALS-associated genes in 60% of FALS and 12% of SALS cases. Double mutations were observed in 5 FALS (2.8%) and 2 SALS (0.4%) individuals. Of these, three carried homozygous or compound heterozygous variants in TARDBP, while four had

heterozygous mutations in two different genes (TARDBP-ANG, TARDBP-c9orf72, SOD1-ANG, and UBQLN2-OPTN). It must be noticed that while the pathogenic role of several mutations identified in our study has been already firmly established, other variants display reduced penetrance in our families, and may thus represent rare benign polymorphisms. Moreover, the phenotype of Italian ALS patients carrying double mutations did not differ significantly from those harboring one or no mutations. As such, it is not possible at present to predict the individual contribution of each variant to ALS pathogenesis in our patients.

P.75. Project MinE: Study design and pilot analyses of a large-scale whole genome sequencing study in ALS

Project MinE Consortium

We recently showed a disproportionate contribution from low-frequency variants in genetic susceptibility to amyotrophic lateral sclerosis (ALS). We therefore have begun Project MinE, an international collaboration that seeks to whole-genome sequence 15,000 ALS patients and 7,500 controls. Here, we report on the design of Project MinE and pilot analyses including 1,264 ALS patients and 611 controls. In the high quality whole genome sequencing data, we find an abundance of rare genetic variation (allele frequency < 0.1%), of which the vast majority is absent in public data sets. Principal component analysis and identity-by-descent (IBD) analyses reveal strong geographical clustering of rare variants and excess of IBD sharing. We further test the implications of poor geographical matching of cases and controls and externally sequenced controls, selected from the identical population as cases. Both approaches can induce false positive associations and reduce power to discover true genetic signal. These results inform us how to optimize the study design of project MinE in order to maximize our ability to find new ALS risk genes.

P.76. NEK1 mutations in familial ALS

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A previous study (Cirulli et al. 2015) showed a suggestive association of NEK1 (NIMA-related kinase) mutations with disease in a cohort of mostly sporadic amyotrophic lateral sclerosis patients. Our analysis of whole exome sequence data from 265 familial ALS patients and 827 controls demonstrated an enrichment of heterozygous NEK1 loss-of-function (LoF) variants in the patient group, while segregation analysis and the high prevalence of NEK1 LoF variants in controls suggest a low penetrance of NEK1 mutations. Additionally, neuropsychological testing and cMRI imaging revealed temporal atrophy and functional hippocampal deficits in a NEK1 LoF mutation carrier, possibly representing a specific phenotype associated with NEK1-linked ALS.

P.77. Molecular diagnosis of Amyotrophic Lateral Sclerosis by Next Generation Sequencing (NGS) analysis of a French cohort of patients

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INTRODUCTION. Amyotrophic Lateral Sclerosis (ALS) is an adult neurodegenerative disease characterized by the progressive death of motor neurons. 5 to 10% of ALS cases are familial, and the study of these families led to the involvement of more than 30 genes. Molecular diagnosis of familial cases of ALS requires an analysis of all these genes. This is now possible through targeted resequencing using Next Generation Sequencing (NGS). The panel of genes to study was chosen in consultation with the French network of ALS Centers and FILSLAN (French rare diseases Healthcare Network: Amyotrophic lateral sclerosis and rare motor neuron diseases). METHODS. ALS patients included in this study showed no expansion of the hexanucleotide repeat in C9ORF72 gene, the most common mutation in ALS. DNA were subjected to enzymatic digestion and hybridization with oligonucleotide probes corresponding to a panel of 33 genes. The libraries, consisting of regions corresponding to exons and intron/exon junctions of all genes (Haloplex, Agilent), were qualified and quantified, and analyzed on a Miseq sequencer (Illumina). RESULTS. We sequenced 33 genes (295 Kb analyzed) in a cohort of French ALS patients. Several sets of 4 and 12 subjects were studied; the number of patients per set was function of the selected flow cells used for sequencing (Illumina). We included in our protocol a double purification of the libraries to remove the dimers of adapters before paired-end sequencing (150bp x 2). The addition of this step has resulted in 90% of reads with a quality above Q30. We used the algorithm Tophat to obtain better alignments on the complete human genome (92% matches on average). Mutations were identified by the software SureCall and then annotated with the Alamut Batch software. Among the 48 mutations identified in the cohort, 23% were listed in the ALSOD database, 42% had allele frequencies below 1% in 1000 Genomes Project, and 13% were new not described to date. All these mutations were confirmed by Sanger sequencing and analyzed by pathogenicity prediction software. DISCUSSION. The molecular diagnostic approach using NGS on familial forms of ALS has become necessary because of the large number of genes involved in the disease, a list of genes that continues to grow. The interest of NGS in ALS is also reinforced by recent results supporting the co-existence of several mutations in a same patient. This approach is therefore justified by an oligo- or polygenic nature of ALS and by phenotypic variations in families, suggesting the involvement of genetic modulators (risk or protecting factors).

P.78. PLCM-seq for robust and efficient transcriptomic profiling of mouse and human motor neurons

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Laser capture microscopy (LCM) coupled with global transcriptome profiling could enable precise analyses of cell populations without the need for tissue dissociation, but has so far required relatively large numbers of cells. Here, we introduce a robust and highly efficient strategy for LCM coupled with full-length mRNA-sequencing (LCM-seq) developed for single-cell transcriptomics. Fixed cells are subjected to direct lysis without RNA extraction, which both simplified the experimental procedures as well as lowered technical noise. We applied LCM-seq on motor neurons isolated from mouse tissues and human post-mortem tissues, and surveyed its performance down to single captured cells. Importantly, we demonstrated that LCM-seq could provide biological insight on highly similar neuronal populations, including motor neurons isolated from different levels of the mouse spinal cord.

P.79. Genetic Background of C9orf72 Expansion and Epigenetic Modifications in Turkish ALS Cases

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The GGGGCC expansion in the C9orf72 gene is not only the most common, but also the first and only dynamic mutation linked to both sporadic and familial forms of ALS resulting in a large clinical heterogeneity ranging from ALS to frontotemporal dementia (FTD). Adding up to the heterogeneous nature of ALS, the clinical variety observed among expansion carriers makes the targeting of the pathogenic mechanisms, underlying different phenotypes, challenging. In the post-genomic era, complex disorders are studied at different levels in order to understand the underlying mechanisms involved in the onset and progression of the disease. Here we aim to investigate Turkish patients carrying the C9orf72 mutation from different angles to make correlations between clinical subtypes. We have performed exome sequencing on 33 C9orf72 expansion carriers to unravel double mutations and additional disease modifiers. Several studies show that hypermethylation is a common phenomenon for repeat expansion disorders like FRDA, Fragile X and myotonic dystrophy, acting as an epigenetic modifier of the disease. The impact of epigenetic modifications on the C9orf72 gene has also become evident; however whether these modifications result in pathogenicity or act as disease modifiers is not clear. In this study, we are also investigating the promoter methylation levels of 44 ALS, six ALS-FTD, two FTD cases and the same number of matched controls using bisulfite conversion coupled with Sanger sequencing. So far among 40 carriers of the C9orf72 expansion, eight ALS patients and one ALS-FTD patient displayed a methylated CpG, only two of them showing hypermethylation scattered to almost all 26 CpG sites. The results of bisulfite sequencing will be validated with restriction enzyme digestion and the effects of hypermethylation on C9orf72 mRNA levels will be observed by qPCR.

P.80. FUS regulates splicing of minor introns: Implications for ALS

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Fused in sarcoma (FUS) is a ubiquitously expressed RNA binding protein proposed to function in various RNA metabolic pathways, including transcription regulation, pre-mRNA splicing, RNA transport, and microRNA processing. Mutations in the FUS gene were identified in patients with amyotrophic lateral sclerosis (ALS), but the pathomechanisms by which these mutations cause ALS are not known. We show that FUS interacts with the minor spliceosome constituent U11 snRNP, binds preferentially to minor introns and directly regulates their removal. Furthermore, a FUS knockout in neuroblastoma cells strongly disturbs the splicing of minor intron-containing mRNAs, among them mRNAs required for action potential transmission and for functional spinal motor units. Moreover, an ALS-associated FUS mutant that forms cytoplasmic aggregates inhibits splicing of minor introns by trapping U11 and U12 snRNAs in these aggregates. Collectively, our findings suggest a possible pathomechanism for ALS in which mutated FUS inhibits correct splicing of minor introns in mRNAs encoding proteins required for motor neuron survival.

P.81. Causes and consequences of microRNA dysregulation in ALS

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The genetics of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia turned our attention to RNA metabolism, primarily because many of the identified diseases-associated genes encode for RNA-binding proteins. microRNAs (miRNAs) are endogenous noncoding RNAs that play critical roles in maintaining brain integrity. Our current studies sheds light on miRNA dysregulation in ALS. We propose that miRNAs are susceptible to fail because protein factors that are critical for miRNA biogenesis are driven to engage in stress-induced abnormal interactions. We further demonstrate how the activity of specific miRNAs controls pathways that are essential for neuronal survival or function and map the new regulatory networks. Taken together, dysregulation of Dicer or the impaired activity of specific miRNAs highlights interesting new target for therapeutic intervention in ALS.

P.82. ALS leads to loss of the TDP-43 repressive function on nonconserved cryptic exons in CNS locations

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One of the fundamental questions since the discovery of TDP-43 aggregates in central nervous system from ALS patients is the contribution of its aggregation and /or loss of function in ALS pathophysiology. Recent data demonstrate a novel role of TDP-43 in the splicing of non-conserved (cryptic) exons from specific mRNAs in both motor cortex and middle temporal gyrus from ALS-FTD patients. In this work, we setup a quantitative method (qPCR) for measuring the relative abundance of the ATG4B and GPSM2 cryptic exons respect to their normal mRNAs in central nervous system. We validated the procedure in samples from different nervous system locations from ALS patients (n=10, mean age=65.7) and age-matched controls (n=10, mean age=73.6). The relative abundance of ATGB cryptic exons in ALS patients was 0.02-0.31% and 0.003-0.74% in spinal cord and motor cortex, respectively, and it is below detection limits in controls. GPSM2 cryptic exons abundances in spinal cord and motor cortex were 0.18-1.58% and 0.08-3.68% in ALS patients, and undetectable and 0-0.24% in controls, respectively. There were significant correlations (R ranging between 0.37 and 0.74, p range 0.0007 to <0.0001) between TDP-43 loss of function in both locations for both genes examined. In addition, the percentages of cryptic exons in spinal cord correlated positively (R = 0.22, p=0.0151 for ATG4B and R=0.55, p<0.0001 for GPSM2) to donor's age, suggesting an interaction with aging. On the other hand, no correlation was observed between relative abundance of ATG4B in motor cortex and age, whereas GPSM2 showed a weak negative correlation (R=0.13, p=0.0434). In conclusion, this method could be useful to specifically quantify TDP-43 loss of function, therefore contributing potentially to ALS diagnoses and stratification.

P.83. A target NGS approach to clarify the role of genetic variants in ALS pathogenesis

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In recent years, with the development of high throughput methods for genetic analysis, the number of genes accounted for being involved in the etiology of amyotrophic lateral sclerosis have been steadily increasing. Consequently, simultaneous screening of multiple genes is likely to be more efficient than gene-by-gene testing in order to identify possible causative variants in ALS patients. Aiming

to investigate the relative impact of genic variants in our cohort of patients, we designed a custom panel of 32 genes (including also both 5' and 3' untranslated regions) and performed targeted next-generation sequencing by using the HaloPlex Target Enrichment System (Agilent) on an Ion Torrent platform (Thermo Fisher Scientific). In parallel, we also applied RP-PCR and standard microsatellite analysis to screen for C9orf72 and ATXN2 repeat expansions, respectively. We will report data of 200 consecutive ALS patients (both familial and sporadic). We could identify variants already known to be responsible for ALS in overall 10 % of cases (including C9orf72 expansion, FUS, SOD1, TARDBP, TBK1 and VCP variants). We found rare coding variants (minor allele frequency < 0.1 %) in any of the 32 tested genes in 17 % of cases, and novel variants in another 20 % of cases. We also detected a series of 5' and 3'-UTR variants. Our panel has been proving to be a useful tool for evaluating and defining the relative contribution of those genes, the effective role of specific coding variants and the possible involvement of variants in 5' and 3' UTRs in ALS. Furthermore, the integration of all genetic data we obtained could allow us to explore the oligogenic hypothesis in ALS etiopathogenesis, considering that we found multiple variants in most of our studied patients.

P.84. A pedigree discordant for p.A4V SOD1 mutation and a novel p.E46D OPTN missense mutation

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Objectives We describe a pedigree with 13 ALS cases, including an affected member and a healthy subject carrying a p.A4V SOD1 mutation and an affected individual with a novel heterozygous OPTN missense mutation not carrying the p.A4V mutation. Methods We analyzed a 33-gene panel applying Ion Torrent PGM next-generation sequencing technology in a familial ALS case who resulted negative for mutations in SOD1, FUS/TLS, TARDBP, c9orf72. Sanger sequence confirmation was performed. Polyphen and MutationTaster were performed for novel genetic variants. Results The proband is 60 year-old man diagnosed with probable spinal-onset ALS. The maternal family included 12 ALS cases, mostly with a rapid course (8-18 months). The only living affected relative developed spinal-onset ALS at 39 and carried a p.A4V SOD1 mutation. Two 65-year healthy relatives showed an affected mother and an affected daughter. One of them agreed to undergo genetic testing and resulted positive for the p.A4V SOD1 mutation. The proband resulted negative for the p.A4V SOD1 mutation. Next-generation sequencing revealed a novel p.E46D heterozygous OPTN missense mutation, leading to the substitution of glutamate with aspartate. It resulted deleterious in silico and absent in our control sample (n= 206). Discussion In our kindred, one healthy and one affected subject carry the p.A4V SOD1 mutation, and an affected member shows a novel p.E46D heterozygous OPTN missense

mutation without SOD1 mutations. Some ALS pedigrees discordant for SOD1 mutations have been already reported and recent studies have demonstrated multiple mutations in several ALS-related genes in sALS and fALS patients, supporting oligogenic inheritance in ALS. We could not evaluate the p.E46D heterozygous OPTN missense mutation recurrence in other relatives, except for the two p.A4V carriers, who were negative. The p.E46D OPTN mutation might contribute to the genetic susceptibility in our patient. Nevertheless further studies are necessary to confirm its possible pathogenic role.

P.85. A novel p.Leu106fs*15 SOD1 mutation with absence of the mutated protein: a case report

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Background. SOD1 mutations account for ~12% of familial ALS cases and ~1% of apparently sporadic (sALS) cases. We report a sALS patient carrying a novel SOD1 mutation (c.320_321insT), leading to a frameshift and the onset of a premature stop codon (p.Leu106fs*15). Case report. The proband is a woman with bulbar onset at the age of 64 and a slowly progressive course. Four years after the onset she displayed walker-assisted gait and required nocturnal non-invasive ventilation. Family history resulted negative for neurologic conditions. We isolated Peripheral Blood Mononuclear Cells (PBMC) and performed Western Immunoblot (WI) with antibodies against full-length SOD1 and against the N-terminal region. The intensity of the wild type SOD1 band in the patient carrying the frameshift mutation was ~50% of the mean intensity detected in three neurologically unaffected subjects, in three sALS subjects without genetic mutations and three ALS patients carrying different SOD1 missense mutations (p.D109Y, p.T137A, p.I113F). In the index case, no band corresponding to the expected molecular weight of the protein encoded by the mutated allele was detected. Total RNA was extracted, retrotranscribed and amplified from lymphocytes of the proband and from a healthy person and a patient carrying a p.D109Y SOD1 missense mutation. The amount of amplified products obtained from the index case was comparable to those obtained from the other two subjects. The sequencing of the amplified products confirmed that in proband's cells the mRNAs corresponding to the two SOD1 alleles are expressed at similar levels. Discussion. Based on such results, the absence of the truncated form of SOD1 protein in the index case is probably due to instability and degradation of the mutated protein. Our findings suggest that this novel SOD1 mutation may be pathogenic through a mechanism of haploinsufficiency.

Mechanisms of Disease

P.86. Understanding the mechanism of SOD1 misfolding - implication to ALS pathogenesis

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A prominent ALS pathogenic protein is a ubiquitous enzyme superoxide dismutase 1 (SOD1) which is responsible for a significant fraction of familial and probably sporadic cases. SOD1 misfolds and eventually aggregates but the nature of the toxic intermediate(s) in the aggregation pathway and its mechanism of pathogenesis remain unknown. A viable possibility is the gain-of-interaction, in which SOD1 forms aberrant interactions with a variety of cellular proteins, hence interfering with their normal function. The ability to form complexes with structurally diverse proteins is a characteristic of proteins whose surface contains highly adaptable energetic hot-spots. The gain-of-interactions of misfolded SOD1 may indicate that some elements of the SOD1's surface acquire certain requisites of the hot-spots. In our study, we characterized the backbone dynamics landscape of the SOD1 surface to pinpoint areas predisposed to aberrant protein-protein interactions (PPI) and identified a SOD1-derived peptide, SE-12, which both induced misfolding and inhibited amyloid formation by mutant SOD1 proteins, redirecting the aggregation pathway toward the formation of amorphous aggregates. The peptide corresponds to the beta-6/beta-7 loop region of SOD1, which was hypothesized to serve as an aggregation-initiating region in misfolded SOD1. Unveiling the modality of peptide SE-12 action and the structural basis of SE-12 interaction with SOD1 is expected to shed light on the identity of the SOD1 noxious species and provide insights into the mechanism of SOD1 misfolding and the propagation of the noxious misfolding signal among structurally intact SOD1 proteins. The fundamental principles underlying the mechanism of transformation of native proteins into noxious ones may be similar for various "aggregation" diseases; therefore, its understanding may pave a way for new strategies to treating these currently intractable diseases.

P.87. UNEXPECTED ROLE OF NUCLEAR SUPEROXIDE DISMUTASE 1

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Background. Over-expression of SOD1 mRNA was recently found in PBMCs from sALS patients (1), despite the unchanged cytoplasmic level of the protein (2). We suppose that the "missing" protein re-localize in the nucleus. In fact recent study showed that, in PBMCs of sALS patients, SOD1 translocates from the cytoplasmic compartment to the nucleus (3). Objectives. We aim to study whether and how SOD1 translocates in the nuclear compartment. We investigate if nuclear SOD1 act as transcription factor in order to protect from oxidative stress in a cellular model of neurodegeneration, neuroblastoma cell line SHSY5Y. Materials and Methods. We studied by both western blot (WB) and immunofluorescence SOD1 localization in sALS patient and in SHSY5Y. By means Mass Spectrometry (MS) we searched for post-translational modifications that permit SOD1 re-localization. Binding protein involved in regulation of SOD1 localization was identified by immunoprecipitation (IP). We analyzed level of histones acetylation by WB. Results. First, we confirmed the nuclear translocation of SOD1 under oxidative stress; SOD1 levels decreases after 30 min of 1mM H2O2 treatment. MS data suggested that SOD1 nuclear re-localization is due to phosphorylation, in which the kinase enzyme Chk2 seems to play a critical role. Finally we found an increase of acetyl-H3, while no changes were observed in the other histone (H2A, H2B and H4). Discussion and Conclusion. We demonstrated that under oxidative stress SOD1, that re-locates at nuclear compartment probably thanks to its phosphorylation by Chk2, is involved in the protection against DNA damage. More interestingly, nuclear SOD1 seems to be involved in the regulation of gene transcription, mainly of those protecting the cell from oxidative stress damage. References. 1) Gagliardi S et al. Neurobiol Dis 2010; 39(2):198-203. 2) Cova E et al. Neurosci Lett 2006 22; 399(3):186-90. 3) Cereda C et al. PLoS One 2013 14; 8(10):e75916.

P.88. THE HSPB8-BAG3-HSP70 COMPLEX MAINTAINS STRESS GRANULE INTEGRITY AND DYNAMISM

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Stress granules (SGs) are ribonucleoprotein complexes induced by stress. They sequester mRNAs and disassemble when the stress subsides, allowing restoration of translation. In amyotrophic lateral sclerosis (ALS) aberrant SGs cannot disassemble, accumulate and can be degraded by autophagy. Indeed, it has been hypothesized that cells prevent the accumulation of aberrant SGs by targeting them for degradation by autophagy (Buchan et al., 2013). However, it is unclear whether autophagy is the preferred pathway of SG clearance or whether there are other ways of dissolving aberrant SGs. Moreover, what are the molecular events causing the formation of aberrant SGs and what are the molecular players regulating this transition are largely unknown. Here, we report evidence for a chaperone-mediated protein quality control system that monitors SG composition and dynamics. We found that defective ribosomal products (DRiPs) accumulate in SGs and affect their dynamic behaviour. We show that only a minor fraction of aberrant SGs is targeted by autophagy, while the vast majority disassembles in a process that requires assistance by the HSPB8-BAG3-HSP70 chaperone complex. Failure of this protein quality control system to prevent accumulation of misfolded proteins inside SGs is the key molecular event that leads to the formation of aberrant SGs and their persistence. Based on our results, we propose a system of chaperone-mediated SG surveillance that regulates SG

composition and dynamism, preventing SG persistency. Quality control of SGs operated by chaperones might be particularly important in (motor neuron) degenerative diseases, such as ALS that are characterized by aberrant SG dynamics.

P.89. TGFβ1 effects in ALS muscle and motorneuron

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Mutations in human superoxide dismutase 1 (SOD1) cause about 20% of inherited cases of fALS. Many evidence suggest that SOD1 toxicity is non-cell autonomous, involving multiple cell types as motorneurons, glial cells and muscle cells. In particular, muscle might be a primary source of toxicity. Transforming growth factor β1 (TGFβ1) has a relevant role in the survival and maintenance of nervous system cells, while in muscle TGFβ1 is involved in development, maintenance and regeneration. Interestingly, TGFβ1 levels are increased in ALS patient serum. Therefore, we evaluated the gene expression of TGFβ1 and its main transduction pathway (which involves Smad proteins) in muscle and spinal cord of Tg ALS mice at different stage of disease . First of all we analysed skeletal muscles of Tg ALS male and female mice expressing mutated hSOD1 at symptomatic and also pre-symptomatic stage, where we observed an increase of TGFβ1 mRNA levels only in male; We found also a decrease of Smad2 and 4, while the Smad3 mRNA levels were increased in both sexes. On the contrary, TGFβ1 expression is decreased in the spinal cord at the pre-symptomatic stage and we observed a decrease of Smad2 and 4 only in female, without variation in Smad3. The high levels of TGFβ1 expression could be related to an increase of muscle fibrosis, while in spinal cord the decrease of expression could result in a lack of neuroprotection in motorneurons. All these results suggest that TGFβ1 could be used as a biomarker of ALS progression in both sexes Grants: ARISLA, Telethon-Italy, Telethon-France, Fondazione Cariplo, AFM, JPND.

P.90. TDP43 inclusions are re-routes to autophagy by the activity of the small chaperone HspB8

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TDP43 is one of the most important protein involved in the pathogenesis of amyotrophic lateral sclerosis (ALS). In ALS patient neurons TDP43 mislocalizes in the

cytoplasm forms inclusions that contain also two fragments of TD43 of 35 and 25 kDa. The presence of these fragments is linked to ALS pathogenesis. In these study we analyzed TDP43 and its disease associated fragments of 35 and 25 kDa in NSC34 cells. By immunofluorescence we observed that TDP25 fragment had a higher clearance than the full length form, but when unsufficiently removed it formed large inclusions in the cytoplasm that colocalized with p62 bodies. In Filter Retardation Assay TDP43 fragments were not detectable due to their higher clearance, while in Western Blot we detect a higher quantity of SDS soluble TDP25. We isolate TDP25 insoluble inclusion using a detergent with a higher solubility power than PBS. By a NP40 extraction we found that most of the TDP25 was detected in the NP40 insoluble fraction. Inhibition of degradative systems with MG132 and 3MA showed that all the three forms of TDPs were degraded by the proteasome while only TDP25 was degraded by autophagy. HspB8 is a protein involved in the cargo delivery to autophagy. It forms a complex with Hsp70 and Bag3 that drives the aggregates to p62 that insert them in autophagosomes. As TDP25 colocalized with p62 we hypotized that the aggregates could be reroutes to autophagy by this pathway. We overexpress HspB8 and found that it greatly reduce NP40 insoluble fraction of TDP25. In conclusion we found that autophagy is involved in removing aggregates of TDP25 fragments and that facilitating autophagy by overexpressing HspB8 greatly reduced the accumulation of ALS associated TDP43 fragments. AFMTELETHON; FONDAZIONE TELETHON; FONDAZIONE CARIPLO; FONDAZIONE ARISLA; Ministero della SanitÃ ; Joint Programme Neurodegenerative Disease (JPND)

P.91. TDP-43 sequestration and aggregation reflects its loss of function also at the proteome level

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TDP-43 protein plays an important role in regulating transcriptional repression, RNA metabolism, and gene splicing. Typically it is shuttled between the nucleus and cytoplasm to perform its functions, but abnormal cytoplasmic aggregation has been associated with neurodegenerative diseases. Aggregation of TDP-43 is most probably the root cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). For the purpose of this study we selected a set of proteins that are misregulated following silencing of TDP-43 and analysed their expression in a TDP-43-aggregation model cell line HEK293 Flp-in Flag-TDP-43-12x-Q/N F4L. Following TDP-43 sequestration in insoluble aggregates, nuclear levels of HNRNPA3 and POLDIP3Î² increased, whereas nuclear levels of PABPC1 and POLDIP3Î± decreased. Predominantly nuclear protein, ALYREF, was translocated to the cytoplasm, and quantities of cytoplasmic protein RANBP1 dropped. In addition, immunofluorescence signal intensity quantification revealed increased nuclear expression of HNRNPL, YARS, and TIAL1, and downregulation of cytoplasmic DPCD. RANBP1 E5 and POLDIP3 E3 were spliced in SH-SY5Y and HEK Flp-in cells with normal TDP-43 levels, whereas when TDP-43 was silenced/aggregated the exons were not excised. This study shows that through endogenous TDP-43 aggregation and sequestration HEK293 Flp-in Flag-TDP-43-12x-Q/N F4L cell line leads to TDP-43 loss of function-like properties also at the proteome level. Further studies need to be conducted on these proteins in ALS/FTLD patient derived samples to elucidate the in vivo distribution.

P.92. TDP-43 post-translational modifications: what role for SUMOylation?

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TDP-43 protein represents the major component of the pathological cytoplasmic inclusions observed in ALS affected brains. The molecular events that lead to TDP-43 aggregate formation are not clear yet. Post-translational modifications, including ubiquitination, phosphorylation and acetylation, are associated with TDP-43 in ALS brains and seem to influence TDP-43 protein aggregation. Differently, SUMOylation of TDP-43 protein has not been investigated yet. A previous study suggested that a splicing isoform of TDP-43 could be conjugated to SUMO-2/3 proteins in the insoluble fraction, nevertheless whether and where TDP-43 is SUMOylated is still unknown. SUMO conjugation controls a variety of biological activities, including nucleocytoplasmic transport, protein stability and aggregation. Therefore, defining SUMOylation role is of critical relevance to understand whether this modification can regulate TDP-43 function and aggregation in the cytoplasm. We tested the hypothesis that TDP-43 is a substrate of SUMOylation by immunoprecipitation (IP) assays in human neuroblastoma cells. Our data show that TDP-43 is conjugated to SUMO-1 and that TDP-43 SUMOylation levels can be modulated upon transfection of plasmids encoding for UBC9 (E2-conjugation enzyme) and SENP1 (deSUMOylation enzyme). To define TDP-43 SUMOylation sites we performed a computational prediction by using bioinformatics algorithms. The analyses highlighted that Lysine136 has the highest prediction score, and that the 106-110 aminoacidic region likely represents a SUMO-interacting motif. By using different TDP-43 deletion mutants we confirmed that the SUMOylation site is comprised within the RRM1 domain. The analysis of alternative splicing of known TDP-43 target transcripts (POLDIP3, TNIK, SEPT6) in condition of SUMO1/UBC9 over-expression showed that SUMOylation regulates TDP-43 splicing activity. Our results indicate that SUMOylation, as a modifier of TDP-43 protein, deserves further investigation as a potential mechanism regulating TDP-43 function and/or aggregation in the cytoplasm in ALS.

P.93. TDP-43 and FUS are exported from the nucleus independent of Exportin-1/CRM1

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TDP-43 (TAR DNA-binding protein of 43 kDa) and FUS (Fused in sarcoma) are nuclear DNA/RNA-binding proteins that play a key role in ALS (Amyotrophic lateral sclerosis) and FTD (Frontotemporal dementia). In ALS and FTD patients both proteins accumulate in insoluble cytoplasmic inclusions, and it has been shown that defects in nuclear import critically contribute to this pathology. As intranuclear inclusions of TDP-43 and FUS are also occasionally found in ALS and FTD patients, it can be speculated that defects in nuclear export of TDP-43 and FUS might also contribute to the pathogenesis of ALS and FTD. However, the mechanisms of nuclear export of TDP-43 and FUS are currently unknown. In this study we focus on the identification

of nuclear export pathways of TDP-43 and FUS. Using the interspecies heterokaryon assay as a nuclear export assay, we could show that both TDP-43 and FUS undergo rapid nucleocytoplasmic shuttling. Bioinformatic predictions identified two putative CRM1/Exportin 1-dependent nuclear export signals (NESs) in both proteins. However, neither mutagenesis of these predicted NESs nor the CRM1-specific inhibitor leptomycin B impaired nuclear export of TDP-43 and FUS, demonstrating that export of TDP-43 and FUS is CRM1-independent. Furthermore, mutation or deletion of various functional domains of TDP-43 and FUS did not inhibit nuclear export of both proteins. Currently, we are using siRNA-mediated knockdown of different export factors (e.g. Exportins or mRNA export factors), in order to test which alternative export factors may mediate nuclear export of TDP-43 and FUS.

P.94. Synthetic peptides prevent the mitochondrial dysfunction in Amyotrophic Lateral Sclerosis

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Over 160 Cu/Zn Superoxide Dismutase (SOD1) missense mutants associate with 20% of familial Amyotrophic Lateral Sclerosis (ALS) cases, forming toxic aggregates onto mitochondrial surface and promoting degeneration of motor neurons mitochondria in spinal cord. The Voltage Dependent Anion selective Channel isoform 1 (VDAC1) is the main channel of the outer mitochondrial membrane (OMM) with a crucial action of gate for metabolic and energetic substrates of the organelle. Moreover, VDAC1 represents the physiological receptor for Bcl-2 proteins and Hexokinases. Literature suggests that VDAC1 acts as a docking site for the mutant SOD1 (mSOD1) in ALS affected motor neuron and this interaction strongly affects the pore-gating properties of VDAC1. Our results confirm that the recombinant SOD1 G93A mutant, but not WT, specifically binds VDAC1 with high affinity and modulates the channel conductance of reconstituted VDAC1 on artificial membrane (Planar Lipid Bilayer). To counteract the mSOD1-VDAC1 toxic interaction, a set of synthetic peptides mimicking the contact surfaces between the two proteins and based on VDAC1 or VDAC1-interactors sequences was produced and the effect of each peptide was tested by different approaches. Our results indicate that the specific peptide H18, upon which a patent is pending (application number: 102016000026259), significantly interferes with in vitro mSOD1-VDAC1 interaction and prevents the toxic SOD1 G93A aggregation on the surface of intact purified mitochondria. Confocal microscopy shows that the H18 peptide expressed in the ALS cell model NSC-34 co-localizes with mitochondria. In addition, suitable cell viability tests underline the ability of H18 peptide to significantly recover cell death of NSC-34 cells overexpressing SOD1 G93A mutant. Overall, our results confirm the intrinsic affinity of SOD1 G93A for VDAC1 and support the use of synthetic peptides as promising therapeutic tool in ALS.
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P.95. Novel cellular models of FUS pathology created by targeted modification of the FUS gene

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A subset of familial ALS cases is characterised by FUSopathy - mislocalisation and aggregation of mutant FUS protein in spinal neurons with its concomitant nuclear clearance. Most of the studies on FUS pathology carried out so far used cellular models with transient or constitutive overexpression of human FUS and its disease-linked variants. Although these have been instrumental to establish cellular processes affected by FUS malfunction, abnormally high FUS levels may results in various artefacts. Previously, we have shown that overexpression of certain ALS-linked FUS variants triggers formation of small granules in the cytoplasm, which shared some common properties but were distinct from stress granules. To assess whether expression of cytoplasmically mislocalised protein at the normal cellular levels from the endogenous FUS gene is sufficient to promote formation of such granules, we created a set of novel cellular models with modified FUS gene using CRISPR/Cas9 technology in human neuroblastoma cells. Several cell lines have been created including lines with one or both FUS alleles edited to remove nuclear localisation signal (NLS) and lines with a longer C-terminal truncation, corresponding to a known familial mutation, G466VfsX14. A line with complete inactivation (knockout) of FUS gene expression has also been produced as a model of FUS loss of function. In cells expressing FUS with deleted NLS, the protein is redistributed to the cytoplasm where it is readily recruited to stress granules upon stress exposure. In one of the lines, FUS-positive cytoplasmic granules are formed which possess characteristics similar to granules previously observed in cells overexpressing ALS-linked FUS variants. These data indicate that the deficiency in FUS nuclear import alone, without an increase in cellular protein levels, is sufficient to trigger and maintain spontaneous RNA-dependent aggregation of FUS protein in the cytoplasm.

P.96. Novel cellular models of FUSopathy provide insights into regulation of paraspeckles in ALS

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Recently, compromised function of specific a nuclear body, the paraspeckle, was linked to molecular pathogenesis of ALS. Previous studies from our and other laboratories have demonstrated that FUS, a protein mislocalising and aggregating in a subset of fALS cases, plays a crucial role in the integrity and function of paraspeckles. However, it is still not clear if and how paraspeckle function can be modulated by mutations affecting FUS, which is mainly due to the lack of adequate models. The utility of cell lines with FUS overexpression and knockdown is limited by the fact that FUS is an essential paraspeckle protein and its levels largely define paraspeckle size and number. To overcome this difficulty, we generated new models of FUSopathy using CRISPR/Cas9-mediated editing in neuroblastoma SH-SY5Y cells to delete gene sequences encoding the nuclear localisation signal of FUS. Stable cell lines heterozygous and homozygous for the truncation were produced; a degree of protein redistribution to the cytoplasm in these lines was consistent with the genotype. Following differentiation into neuron-like cells, the Nanostring (nCounter) platform

was employed to assess expression of a number of known FUS target genes as well as genes linked to paraspeckle function. Changes in gene expression consistent with both loss of nuclear function and gain of cytoplasmic function were identified. As expected, prominent FUS mislocalisation seen in homozygous lines as well as knockout of FUS led to loss of paraspeckles. Strikingly, we found that moderate FUS mislocalisation can result in paraspeckle enlargement which is due to compensatory upregulation of other core paraspeckle proteins. Finally, we were able to show paraspeckle formation alongside with upregulation of paraspeckle proteins in spinal neurons of FUS-ALS patients. We suggest that mislocalisation of FUS can trigger accumulation of other RNA-binding proteins and associated paraspeckle formation despite lowered nuclear levels of FUS.

P.97. Neuregulin and ErbB4 receptor abnormalities in Amyotrophic Lateral Sclerosis

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The death of motoneurons in Amyotrophic Lateral Sclerosis (ALS) is preceded by failure of neuromuscular junctions (NMJs) and axonal retraction, dependent upon deffective interaction of motor axons with terminal Schwann cells and skeletal muscle fibers. Neuregulin1 (Nrg1) is a neurotrophic factor highly expressed in motoneurons and NMJs that supports axonal and neuromuscular development and maintenance. Interestingly, Nrg1 expression has been previously found reduced in both ALS patients and SOD1G93A mice. We have characterized the expression of Nrg1 isoforms and ErbB receptors in spinal cord and skeletal muscles of SOD1G93A mice and ALS patients. In SOD1G93A mice skeletal muscles we found a significant reduction of ErbB4 mRNA levels, correlating with the muscle denervation. Skeletal muscles from sporadic and familial ALS patients also showed reduced levels of ErbB4 receptor at protein and mRNA levels. We confirmed that in the spinal cord of the ALS mice, Nrg1 type I mRNA levels increased along the progression of the disease, whereas Nrg1 type III mRNA levels were reduced. We also observed that in ALS mice ErbB4 receptor translocates into the nucleus at the end stage of the disease (16 weeks). Such intranuclear ErbB4 receptor could play a role in the regulation of gene expression. In conclusion, the several abnormalities observed in the Nrg1-ErbB pathway suggest that it is an interesting therapeutical target for improving motoneuron survival and/or NMJ maintenance in ALS.

P.98. 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. FUS is a ubiquitously expressed nuclear protein which, in FUS-ALS, is mislocalized to the cytoplasm and forms aggregates. A cytoplasmic translocation of FUS was suggested as a key event in FUS-ALS pathology, while FUS aggregation is thought to be caused by a second hit. Here we present the first human in vitro model of FUS-ALS that allows the (patho-)physiological investigation of FUS distribution in patient-derived cortical neurons carrying endogenous mutation. We could show that observed pathological phenotypes can be related to clinically early (R495QfsX527) vs. late (R521C) disease onset. We found that the amount of cytoplasmic FUS as well as cellular vulnerability depends on the severity of the underlying mutation. Cytoplasmic FUS inclusions formed spontaneously in mutated iPSC-derived cortical neurons depending both on the severity of FUS mutation and neural aging but independent of a second hit. Finally, neurodegeneration was not specific to layer V cortical neurons, which are mainly affected in ALS. Pathological phenotypes were also seen in layer II/III cortical neurons typically affected in Frontotemporal lobar degeneration (FTLD), even though FUS mutations do not cause FTLD. 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.

P.99. MIF alters misfolded SOD1 amyloid aggregation by inducing the formation of disordered aggregates

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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. Now,

we show by microscale thermophoresis and FRET that MIF directly interacts with misfolded SOD1, and inhibits misfolded SOD1 toxicity in motor neuron-like cells. By using ThT assay coupled with TEM imaging, we show that MIF alters the regular misfolded SOD1 aggregation pathway of fiber-like aggregates, and promotes instead the formation of the less toxic disordered aggregates. In addition, by using different MIF mutants, we show that MIF known enzymatic activities are dispensable for the chaperone-like function and the trimer conformation seems to be the crucial one for its protective effect. We believe this information will lead to a better understanding of the misfolded SOD1 toxicity mechanism, and may contribute for the future development of MIF-based therapies for ALS.

P.100. Intranuclear (GGGGCC)n RNA foci induce formation of paraspeckle-like structures

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Expansion of GGGGCC (G4C2) hexanucleotide repeat in the gene C9orf72 is the most common pathogenic mutation in families with autosomal dominant FTD, FTD/ALS and ALS. Normally, there are <10 repeats in healthy individuals, however, in C9orf72 associated cases hundreds or thousands of repeats can be detected. The expanded repeat is transcribed and can form intranuclear RNA foci. Here we show that the paraspeckle proteins SFPQ, NONO and PSPC1 bind to (G4C2)n RNA in vitro and colocalize with intranuclear RNA foci in cells transfected with expanded repeats. Paraspeckles are nuclear structures that function in nuclear retention of adenosine to inosine edited RNA. They are formed on a backbone of the long non-coding RNA NEAT1. Only a small fraction of G4C2 RNA foci colocalize with NEAT1. Furthermore, the presence of RNA foci increased the number of SFPQ stained subnuclear bodies. This suggests that (G4C2)n RNA may replace NEAT1, leading to formation of paraspecle-like structures, which could cause an increase in the nuclear retention of edited RNA. Our study identifies an additional mechanism through which hexanucleotide expansion may lead to neurodegeneration.

P.101. Enhancing mitochondrial fusion is neuroprotective in TDP-43 Drosophila models of ALS

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Neurons are cells with high energy demands and very sensitive to oxidative stress. Therefore, they are particularly vulnerable to mitochondrial defects. In recent years, mitochondrial dysfunction has been proposed to be implicated in the pathogenesis of several neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS). ALS is a fatal disease characterized by progressive motor neuron degeneration. So far, mitochondrial abnormalities in ALS were mainly studied on mutant SOD1 models, so little is known about the impact of other ALS-related genes on mitochondria. Using Drosophila models, we wish to determine whether mitochondrial dysfunction is a central determinant in ALS pathogenesis. TAR DNA-binding protein 43 kDa (TDP-43), a gene which regulates RNA metabolism, has been recently linked to ALS. Mutations in TDP-43 have been identified in familial ALS cases, and wild-type TDP-43 has been also reported to form neuronal cytoplasmic inclusions in almost all sporadic cases, suggesting that TDP-43 dysregulation can be a common feature in ALS. Using electron microscopy, we discovered that mitochondria in Drosophila neurons overexpressing human wild-type TDP-43 (hTDP-43) are smaller. We found that hTDP-43-induced mitochondrial fragmentation is correlated with a decrease in the mRNA and protein levels of the Drosophila pro-fusion factor Mitofusin. We are currently deciphering the mechanisms responsible of the decrease in Mitofusin expression. More importantly, overexpression of Drosophila Mitofusin ameliorated both induced and spontaneous locomotor defects in hTDP-43-expressing flies. The same results were obtained in a fly model expressing a mutant form of hTDP-43 (G298S mutation). This study highlights the importance of defects in mitochondrial dynamics in ALS pathogenesis. Moreover, we show for the first time that increasing mitochondrial fusion is beneficial in an in vivo model of ALS, serving as a possible therapeutic target against this disease.

P.102. Deciphering the pathological response of the astrocytes in Amyotrophic Lateral Sclerosis

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Collective evidence indicates that motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS) is non-cell-autonomous and requires the interaction with the neighboring astrocytes. Astrocytes can hurt motor neurons by secreting neurotoxic factors, but they can play deleterious roles also by losing functions that are supportive for neurons. Recently, we reported that stimulation of inositol 1,4,5 triphosphate (IP3)-generating group I metabotropic glutamate receptors in ALS astrocytes triggers abundant and persistent elevations of intracellular Ca2+ concentrations in the absence of spontaneous oscillations. This correlates with mitochondrial disarrangement and cell death in subsets of astrocytes. The interaction of IP3 receptors with the anti-apoptotic protein Bcl-XL was previously described to prevent cell death by generating pro-survival Ca2+ oscillations. In ALS astrocytes, we found that the sole BH4 domain of Bcl-XL, fused to the protein transduction domain of the HIV-1 TAT protein (TAT-BH4), is sufficient to restore sustained Ca2+ oscillations and

cell death resistance. Furthermore, chronic treatment of ALS mice with the TAT-BH4 peptide exerts a positive impact on the disease manifestations. Besides, we demonstrate that ALS astrocytes aberrantly respond to a neuroinflammatory microenvironment, exhibiting a functional impairment of their neurotrophic properties.

P.103. Clearance and transport of misfolded protein responsible for motor neuron diseases (MNDs)

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Mutated proteins responsible for motor neuron diseases such as as: mutant SOD1, TDP-43, FUS, VCP, OPTN, and recently C9ORF72 RAN translated poly di-peptide in familial and sporadic ALS amyotrophic lateral sclerosis (ALS) and mutant androgen receptor (ARpolyQ) in spinal and bulbar muscular atrophy (SBMA) misfolds and aggregates into cytoplasm or nuclei of motor neuron. The protein quality control (PQC) system maintains protein homeostasis by re-folding (by chaperone) or by degradation (by autophagy or proteasome) of misfolded proteins. This prevents their toxicity. Here the trafficking of mutated misfolded proteins represents a key point to control their aggregation and degradation. In NSC34 cells, we evaluated the role of dynein retrograde transport on the PQC. Using RT-qPCR, WB and IF analysis we observed that dynein down-regulation altered SQSTM1/p62 and LC3 localization. Similarly, dynein ATPase activity inhibition by EHNA compound counteracted the induction of SQSTM1/p62 and LC3 levels by trehalose treatment. In addition, dynein inhibition increased selectively BAG1 mRNA involved in misfolded protein degradation via proteasome. Indeed, dynein inhibition clearly reduced the PBS insoluble fraction of mutated misfolded proteins (SOD1-G93A, TDP-43 C and ARpolyQ) in filter retardation assay (FRA) also when autophagy was blocked while its effects was counteracted by proteasome inhibition. In addition, BAG1 overexpression reduced aggregation of misfolded species and BAG1 down-regulation blocked the EHNA effects. Interestingly, we observed that C9ORF72 poly-GP insoluble species were processed by autophagy via HSPB8 facilitation and also in this particular model dynein inhibition counteracted the formation of misfolded protein insoluble species. Collectively, these data showed that when autophagy alteration occurs misfolded proteins can be re-routed to proteasome for degradation by BAG1. GRANTS: AFM-TELETHON; FONDAZIONE TELETHON; FONDAZIONE CARIPLO; FONDAZIONE ARISLA; Ministero della SanitÃ ; Joint Programme Neurodegenerative Disease (JPND)

P.104. Characterization of physiological and pathological functions of HECW1 in ALS

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Intraneuronal aggregates of insoluble ubiquitinated proteins are a major pathological hallmark of many neurodegenerative diseases. The effective role (protective or toxic) of these inclusions as well as the molecular mechanisms underlying their ubiquitination process, have been elusive. How proteins within these inclusion bodies escape proteasomal degradation despite being enriched with ubiquitin (Ub) remains an unresolved conundrum. Their stability may be due to decreased levels of proteasomal activity, which is associated with increasing age. Alternatively, the formation of Ub-enriched inclusions may be uncoupled from the proteasome as a consequence of the linkage-specific Ub chains attached to the proteins. HECW1 is a poorly characterised Nedd4-family E3 ligase member and it has been involved in familial Amyotrophic Lateral Sclerosis (fALS). Immunohistochemical analysis revealed that HECW1 is localized within the inclusion bodies in the spinal cord of ALS patients and mutated SOD1 transgenic mice. It ubiquitinates mutated SOD1, but does not recognize or modify WT SOD1. Transgenic mice overexpressing HECW1 show loss of neurons in the spinal cord, muscular atrophy and microglia activation, which are common features of ALS. Our initial observations indicate that an activated form of HECW1 generates cellular aggregates where ubiquitin and p62 are re-localized. Validated mass spectrometry data indicate that p62 and RNA processing proteins are HECW1 interactors, suggesting a direct role of HECW1 in selective autophagy and in RNA metabolism, two pathways deregulated in the pathogenesis of ALS. To gain insights in the pathological role of HECW1 in ALS we have generated catalytically inactive alleles of the Drosophila ortholog, the uncharacterized gene CG42797, Mutant animals lived shorter and presented serious locomotive problems, assessed by negative geotaxis assay. Histological analysis of fly brains revealed high vacuolization of the tissue, visible before the appearance of the climbing defect. In addition, we observed a genetic interaction between CG42797 and TBPH (TDP43 ortholog).

P.105. Characterization of TDP-43 splicing target TNIK in neuronal differentiation

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Cytoplasmic TDP-43 inclusions represent the neuropathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTLD), although the pathogenic mechanisms associated to TDP-43 still remain unclear. We have recently demonstrated that TDP-43 regulates alternative splicing of several pre-mRNA targets, including TNIK, a Ser/Thr kinase highly expressed in the brain with an important role in synaptic function, neurogenesis and cytoskeleton dynamics. TDP-

43 negatively regulates the inclusion of TNIK exon 15 which encodes for a 29 aminoacidic sequence in the intermediate domain of the protein. In condition of TDP-43 knock-down, we observed an enrichment of TNIK protein isoforms containing exon 15 (TNIK-ex15) which show a different subcellular localization compared to other TNIK isoforms. Aim of this study was to further characterize the alternative splicing of TNIK gene in human tissues and in in vitro neuronal differentiation models. By RT-PCR we observed that TNIK-ex15 isoforms were enriched in all cerebral and spinal cord regions analysed. This correlated with an increase of TNIK-ex15 protein level by WB analysis using a custom antibody raised against exon15-encoded aminoacidic sequence. We also investigated if TNIK alternative splicing is regulated during neuronal differentiation in human neuroblastoma cells treated with retinoic acidic and in iPSCs induced to differentiate into motoneurons. A significant increase of TNIK-ex15 isoforms was observed both at transcript and protein level in association to neuronal differentiation. Immunofluorescence analyses showed a prevalent perinuclear distribution of TNIK-ex15 protein in differentiated cells. Our data indicate a potential role of TNIK in neuronal differentiation although the specific function of TNIK-ex15 protein isoform needs to be further investigated also in association to ALS/FTLD diseases.

P.106. Cellular stress impairs the physiological function of TDP-43

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TAR DNA binding protein of 43 kDa (TDP-43) is a nuclear DNA/RNA-binding protein that regulates RNA levels and splicing of several hundred target genes. TDP-43 has been linked to neurodegeneration, as mutations in TDP-43 cause familial amyotrophic lateral sclerosis (ALS) and TDP-43-positive neuronal cytoplasmic inclusions are a pathological hallmark of ALS and frontotemporal dementia (FTD). Cellular stress and stress granules have been implicated in the formation these protein deposits, since they frequently co-localize with stress granule marker proteins. Besides cytoplasmic TDP-43 inclusions, intranuclear TDP-43 inclusions are occasionally observed in post mortem brains of ALS and FTD patients, but their origin remains unclear. Moreover, it is unknown how cellular stress affects the physiological function of TDP-43. Here we show that cellular stress, most notably heat shock or heavy metal treatment, causes transient recruitment of TDP-43 into nuclear stress bodies (NSBs), highly packed ribonucleoprotein structures that form in response to cellular stress. Localization of TDP-43 in NSBs requires the C-terminal low complexity domain, but not RNA-binding, suggesting that TDP-43 is recruited to NSBs via the low complexity domain. Nevertheless, ALS-associated point mutations in this domain do not alter recruitment of TDP-43 into NSBs. Interestingly, alternative splicing of several endogenous TDP-43 target genes is impaired when TDP-43 is localized in NSBs, suggesting that the

splicing activity of TDP-43 is transiently disturbed when TDP-43 is recruited into NSBs upon cellular stress. Thus, cellular stress leads to a transient loss of function of TDP-43. Furthermore, it can be speculated that cellular stress may not only be important for the formation of cytoplasmic TDP-43 inclusions, but may also be involved in the formation of intranuclear TDP-43 aggregates.

P.107. Calcium-responsive transactivator protein (CREST) shares common properties with other ALS-associated proteins

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Calcium-responsive transactivator (CREST) is a neurospecific protein which has recently been implicated in amyotrophic lateral sclerosis (ALS). Structurally, CREST consists of a C-terminal transactivation domain, which is characterized by low sequence complexity and satisfies the criteria for a prion-like domain that unify it with other ALS-associated genes. The protein has also been reported to localise to paraspeckles. Several stable lines were used to evaluate aggregation propensity, protein stability, recruitment to stress granules and paraspeckles for normal CREST and its ALS-associated mutants. Mouse hippocampal cultures were used to assess dendritic complexity upon expression of normal and mutant CREST. Transgenic Drosophila models were also produced to test possible in vivo toxicity of CREST. We have shown that CREST is prone to aggregation and is recruited to stress-induced stress granules in cultured cells. Aggregation of CREST affects paraspeckle integrity, probably by trapping other paraspeckle proteins within aggregates. Neither of the four CREST mutations described in ALS alters its subcellular localization, stress granule recruitment or detergent solubility; however Q388stop mutation results in elevated steady-state levels and more frequent nuclear aggregation of the protein. Both wild-type protein and its mutants negatively affect neurite network complexity of mouse cultured neurons when overexpressed, with Q388stop mutation being the most deleterious. When overexpressed in the fly eye, wild-type CREST or its mutants lead to severe retinal degeneration without obvious differences between the variants. Our study presents evidence that CREST has common properties with other ALS-associated proteins implicated in ALS pathogenesis, namely the ability to aggregate, be recruited to stress granules and alter paraspeckle integrity. A change in CREST levels in neurons which might occur under pathological conditions would have a profound negative effect on neuronal homeostasis.

P.108. C9ORF72 rescues the loss of function of the autophagy initiator p62/SQSTM1

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ALS has a major genetic contribution with the most common genetic abnormality being the GGGGCC hexanucleotide repeat expansion (HRE) in the first intron of the C9ORF72 gene (20-50% cases). However, the precise function of C9ORF72 has not yet been determined. One of the hypotheses is a role in autophagy. 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 (within 1 to 3.5%) with a well-known cellular function. SQSTM1/p62, is an essential actor and regulator of the initiation of the autophagy pathway. Inclusions p62+ 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 SQSTM1 and C9orf72 mutations in ALS, we developed zebrafish models for either of these genes. Loss of function of the Sqstm1 and/or C9orf72 zebrafish orthologues leads to a specific motor phenotype associated with shorter motor neuronal axons and reduced swimming capacity. These deficits can be rescued by the overexpression of wild-type human genes. In the Sqstm1 model, there was no rescue by constructs carrying ALS-related mutations and treatment of zebrafish phenotypic embryos with rapamycin, a known activator of autophagy, ameliorates the locomotor behavior. To elucidate the common cellular mechanisms underlying autophagy deregulation in motor neuron degeneration, C9orf72 and Sqstm1 zebrafish models were utilized 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, which is consistent with a probable role of C9orf72 in the autophagy degradation pathway, thus opening novel avenues for potential treatment of ALS.

P.109. Analysis of the hnRNP A family in health and disease in zebrafish

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The causes of Amyotrophic Lateral Sclerosis (ALS) remain largely unknown, especially in the 90% of the sporadic cases. The remaining 10% have a hereditary component (fALS), whereby the majority of mutations are found in genes with RNA-binding function, including TAR DNA Binding Protein (TDP-43) and Fused in Sarcoma (FUS). Both of these proteins are members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family of RNA binding proteins (RBP). Increasing evidence shows the importance of mutations in RBP and impaired RNA metabolism in neurological diseases. Recent studies have linked other members of the hnRNP family to ALS: hnRNP A1, hnRNP A2B1 and hnRNP A3. These proteins were shown to exhibit aggregation potential in disease state and can bind TDP-43 and C9orf72 hexanucleotide repeat expansions. Furthermore mutations in hnRNP A1 and hnRNP A2B1 were identified in a Multisystem Proteinopathy (MSP) family including a case of fALS, and mislocalization of the encoded proteins was observed in muscle tissue from affected patients. Although these proteins have numerous functions in RNA processing, their role in the central nervous system is poorly understood. Mutations

in RBPs might contribute to disease by leading to a loss of protein function resulting in detrimental effects for the cell. Alternatively, the mutation increases the aggregating potential of these proteins and thereby triggers toxicity by sequestering or mislocalizing proteins and RNAs. In this study we model potential pathomechanisms in vivo by generating zebrafish disease models using the CRISPR/Cas9 system. To model physiological loss of function and the consequences of ALS-causing mutations we established knockout lines as well as knock in lines carrying the patient mutations. By overexpressing the mutant and wildtype protein form we additionally investigate their aggregation potential. Altogether, this study aims to identify hnRNP A function in vivo and to thereby further unravel molecular mechanisms underlying ALS.

P.110. ALS-causing missense mutations of CHCHD10 affect protein structure and stability

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Coiled-coil helix coiled-coil helix domain containing protein 10 (CHCHD10) is a mitochondrial protein encoded in the nucleus. It is enriched at the cristae junctions of mitochondria and possibly plays a role in cristae structure, respiratory chain regulation and mtDNA stability. The finding that mutations in CHCHD10 are associated with familial ALS and FTD directly links mitochondrial dysfunction to the pathology of the ALS-FTD continuum. However, the molecular pathogenesis of CHCHD10-related neurodegeneration remained so far enigmatic. In this study we analyze the impact of three missense variants found in ALS patients (R15L, P34S and G66V) on the structure and stability of CHCHD10. To that end, we employ CD spectroscopy, protein half-life determination and Thermofluor assay. The R15L and G66V, but not the P34S substitution, considerably changes the protein structure and decreases the stability of CHCHD10. These results match most recent genetic evidence indicating that the R15L and G66V variants of CHCHD10 are pathogenic while P34S is significantly associated with neurodegenerative diseases. We thus hypothesize that mutations of CHCHD10 induce a structural disturbance and loss-of-function of CHCHD10. Consequently, in order to elucidate downstream pathways mediating detrimental effects of CHCHD10 mutations, we performed a mass spectrometry-based screen for differential, disease-relevant binding partners of wild-type or R15L and G66V mutant CHCHD10, respectively.

Therapy

P.111. The CANALS study: A RCT to Assess Safety and Efficacy on Spasticity of a C. Sativa Extract in MND

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Introduction: Spasticity is a one of the major determinant of functional loss and decline in quality of life in ALS and other motor neuron disease (MND) patients. In recent years, several clinical trials have tested the efficacy of cannabis on spasticity in multiple sclerosis. The study's primary was to evaluate the safety, tolerability and efficacy of a Cannabis Sativa extract medium-term treatment (6 weeks) to improve spasticity in ALS and MND patients. Methods: 60 consecutive patients fulfilling specific inclusion criteria were randomized and double blinded allocated to receive a cannabis extract oral spray or placebo. Primary end-point was improvement in the modified 5- points modified Ashworth Scale (MAS). Secondary End-points: spasticity, spasm frequency and sleep disruption (0-10 NRS score); Function: walking ability, functional scores (ALSFRS-R); pain (0-10 NRS score). The Global Impression of Change (GIC) for the patient, carer and clinician. Results: the study drug was well tolerated, none of the patients included withdrew from the study. We observed a positive trend for improvement of all outcome measures in the active drug arm compared to the placebo group, which reached statistical significance for the MAS mean score (p=0.013) and pain NRS (p=0.013). Patients' GIC demonstrated a significant subjective improvement in 55% of subjects (p=0.001). Conclusions: Our pilot study suggests that cannabinoids may represent a valuable option for spasticity treatment in MND patients. Moreover, it may have also additional beneficial effects such as pain relief. Further studies are needed in order to confirm our results. Acknowledgements: This study has been funded by Fondazione Italiana di Ricerca per la Sclerosi Laterale Amiotrofica (AriSLA, CANALS Project).

P.112. Safety and efficacy of botulinum toxin A for spasticity in amyotrophic lateral sclerosis

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Introduction Spasticity can be a very disabling problem in some amyotrophic lateral sclerosis (ALS) phenotypes, such as upper motor neuron dominant ALS (UMN-ALS) and primary lateral sclerosis (PLS). Our aim is to describe the safety and efficacy of botulinum toxin A (BoTox-A) for improving gait in those ALS phenotypes. Methods UMN-ALS and PLS outpatients, experiencing gait disturbances secondary to moderate to severe spasticity despite optimized oral medication, were offered BoTox-A treatment. Stretching exercises were indicated to complement BoTox-A effect and ankle-foot orthotics (AFOs) were prescribed when appropriate. Tolerance (muscle strength, disease progression rate) and efficacy (10-meter walk test) were measured at baseline and after treatment. Results Eight out of 122 ALS outpatients fulfilled clinical criteria and were offered to participate. One declined. The other seven were administered BoTox-A in the lower limbs, every 5-8 months. All of them experienced improvement in the clinical outcome and all but one referred subjective improvement. Moreover, after a median follow up of 16 months and three injections, BoTox-A effect was maintained with no adverse events. Conclusions This study provides class IV evidence that BoTox-A is effective and safe in the short- and long-term in a subset of ALS patients with moderate to severe spasticity.

P.113. SOD1/Rag2-/- mice with a low copy number of the SOD1 gene as a model for stem cell therapy of ALS

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There is an increasing agreement that glial cells contribute to the premature death of motor neurons - a hallmark of ALS. Thus, global replacement of glial cells is a valid therapeutic strategy. Until now, the positive effects of stem cell therapies in mouse models of ALS have been observed by many groups, but no cure has been achieved. We have already shown that human glial-restricted precursors are capable of replacing a host glia and cure immunodeficient rag2-/- shiverer mice, if transplanted at the neonatal stage. The life-span of untreated shiverer mice is quite long (200 days) thus transplanted cells have over 6 months to migrate and differentiate. Actually, the time from human GRP transplantation to the myelin basic protein expression lasts 3-6 months. We hypothesize that the life-span of most popular high copy number SOD1 mice is only about 130 days, which is too short to realize the full advantages of transplanted stem cells for the global replacement of potentially defective and toxic host glia. Thus, we focused on developing immunodeficient rag2-/-, long-living mice with a low copy number of the SOD1 gene and a longer life-span. The obtained double-mutant SOD1/rag-/- mice have been characterized genetically and behaviorally. Quantitative PCR performed against the standards has revealed that the line of our mice has an average of 1.56 copies compared to 10 copies in typically used SOD1 animals. This translated to a significant increase in life-span from 130 days in wild-type SOD1 mice to 250 days in our double mutants. The death of long-living SOD1/rag2-/- mice is preceded by muscular weakness, as measured by our in-house-developed hind limb test. To conclude, we developed long-living double mutant SOD1/rag2-/- mice, which could be useful for testing therapeutic utility of human stem

cells for a cell replacement approach in the treatment of ALS. Supported by a NCR&D grant for STRATEGMED project: "GRP&ALS;"

P.114. Role of the mitochondrial Na/Ca/Li-exchanger (NCLX) in the pathophysiology of ALS

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Background: Disturbance of the ER-mitochondria-coupling-cycle (ERMCC) appears to be an important feature in the pathophysiology of ALS. This includes ER calcium depletion and overload in mitochondria which take up large amounts of calcium. Calcium release is mediated by the sodium-calcium-lithium-exchanger (NCLX) localized at the inner mitochondrial membrane. It is the main calcium eliminating pathway of excitable cells. As a part of the ERMCC modulating the NCLX can provide a new therapeutical principle. Objectives: to investigate (i) the distribution and (ii) expression level of NCLX, to determine effects of NCLX modulators on (iii) neuronal survival and (iv) ERMCC. Methods: Murine motor neuron co-cultures without (NT) or with (TG) overexpression of hSOD1G93A were used for immunofluorescent staining. Survival assays were performed in presence or absence of CGP35157 (NCLX inhibitor) or forskolin (activator) alone or in combination with kainate for 12h. Calcium imaging with fura-2AM was used to assess changes in intracellular calcium concentrations. Western blot analyses were carried out using SOD1-G93A and SOD1-WT overexpressing NSC34. Results: In western blot basal protein levels of the NCLX in NSC G93A were significantly reduced compared to NSC SOD1 WT. Application of forskolin to the G93A cell line generated a significant upregulation of NCLX protein level. CGP35157 treatment did not show effects on NCLX expression, but modulated ERMCC in motor neurons and improved survival of motoneurons in KA toxicity. Conclusions: ERMCC dysregulation can induce disruptions in different cell processes finally leading to cell death. A dysfunction of NCLX can contribute an impaired ERMCC. Hence NCLX modulation may be beneficial to the viability of motor neurons. Acknowledgment: This research is supported by BMBF (Bundesministerium fÃ¼r Bildung and Forschung) in the framework of the E-RARE programme (PYRAMID) and JPND (SOPHIA) of the European Union.

P.115. Riluzole display glutamate-independent antioxidant properties: relevance for ALS

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Amyotrophic lateral sclerosis (ALS) is a relentless neurodegenerative disease for which, at the moment, no effective therapies exist. Riluzole is the only drug currently licensed for ALS, at the dose of 100 mg/day. Uncertainties regarding the exact

mechanism of action of this drug hinder any attempt of designing more potent drugs able to increase significantly life expectancy in ALS. Riluzole specifically blocks tetrodotoxin-sensitive sodium channels reducing calcium influx and excessive glutamate receptor stimulation within the SNC. However, albeit antioxidant properties of this molecule have already been hypothesized, previous works conceivably did not distinguish between direct and indirect antioxidant effects (the latter due to the known anti-glutamatergic properties). In this work we used a neuroblastoma cell model (SH-SY5Y), initially demonstrating its insensitivity to various excitotoxic insults. In contrast, when these cells were exposed to an acute oxidative insult (H2O2 200 ÂµM/24 h), cell viability was reduced of about 50% and whole-cell ROS were increased three-fold; riluzole co-administration elicited a significant rescue starting from the concentration of 0.5-1 ÂµM. Neuroblastoma cells carrying the SOD1-G93A ALS-related mutation showed a twofold increase in whole-cell ROS production with respect to wild type cells, that was unaffected by riluzole co-administration. In this work we used an excitotoxicity-insensitive model in order to demonstrate that riluzole possesses direct antioxidant properties. However, in our experimental condition, this was not sufficient to protect against SOD1-related chronic damage. These results cast doubts on the potential efficacy of antioxidant molecules in ALS prompting to look for different mechanisms of action for discovering a cure.

P.116. Value of sequential designs for amyotrophic lateral sclerosis clinical trials

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Background. Traditional trial designs use predetermined sample sizes and a fixed follow-up period. Sequential designs permit discontinuation of a study as soon as there is enough evidence for superiority or futility of a treatment. The aim of this study is to determine the value of a sequential design for a phase-III ALS clinical trial. Methods. Data originated from the EMPOWER study, a double-blind, randomized, phase 3 clinical trial to evaluate efficacy of dexpramipexole for ALS. Eighty-one medical centers were involved and enrolled in total 943 participants. Participants were allocated to twice-daily dexpramipexole or placebo for 12-18 months. No treatment effects were found. The trial was redesigned and analyzed according to a sequential design using different interim schedules. The designs were compared with a traditional design in terms of power, expected sample size and trial duration. The primary outcome was the mean difference in ALSFRS-R at 12 months follow-up. Results. The EMPOWER study was based on a sample size of 344 participants per group to detect a 2.13 mean difference in ALSFRS-R at 12 months. A sequential design would increase the maximum sample size with 2.0 - 7.0% or reduce power with -0.5 - -1.63%, depending on the number of interim analyses. The EMPOWER study could have been stopped at the first interim analysis when applying a sequential scheme with four interim analysis, indicating that only 30% of the 12-month outcomes were necessary to show futility of dexpramipexole. The entire duration of the trial could

be shortened with 17.3%, leading to an 18.9% reduction in the number of follow-up visits. Conclusions. Future ALS clinical trials can become more efficient by incorporating sequential analyses schemes. Especially due the current lack of effective therapeutic strategies, stopping futile clinical trials would be very beneficial for ALS research by earlier exploration of further promising treatments.

P.117. Targeting MCU within ERMCC in ALS

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Background: Disruption of ER-mitochondria-calcium-cycle (ERMCC) in hSOD1G93A model of ALS is characterized by Ca2+ depletion of the ER and Ca2+ overload of the mitochondria. Ca2+ is channelled through the inner mitochondrial membrane by the mitochondrial calcium uniporter complex (MCU) and through outer mitochondrial membrane by voltage dependent anion channel (VDAC). In extreme stress situations, as mitochondrial calcium overload, mitochondrial permeability transition pore (mPTP) is formed and the apoptotic cell death is activated due to the release of cytochrome c. Objectives: Our objectives were: to investigate expression of MCU, VDAC and Cyclophilin D (CyPD, modulator of mPTP) in primary nontg and hSOD1G93A mouse motor neurons; influence ERMCC by pharmacological manipulation of mitochondrial calcium uptake as possible rescue strategy. Methods: Immunocytochemistry and RT-qPCR are done to investigate mRNA level and expression of target proteins. For survival assay we challenged our cultures with kainate to induce excitotoxicity and applied KN-62 and kaempherol as possible rescue drugs. Results: MCU was overexpressed in hSOD1G93A motor neurons suggesting involvement of MCU in mitochondrial Ca2+ overload. Decreased immunostaining signal for CyPD was found in hSOD1G93A, possibly as protection from mPTP formation. MCU modulation by CaM kinase inhibitor KN-62 and MCU activator kaempherol protected hSOD1G93A motor neurons from kainite-induced exitotoxicity. Mechanisms underlying protective effects of these two drugs are under investigation. Influence of the drugs on MCU expression and their functional consequences will be investigated by protein quantification and single cell calcium imaging. Conclusion: Not only expression level of MCU is playing a role in pathophysiology of ALS, but also sensitivity of mitochondrial Ca2+ channels to changes in cytosolic Ca2+. Since MCU does not require cotransport to channel Ca2+ into mitochondria, it is possible that the same complex works reversibly too. Deeper inside in regulatory subunits of MCU is necessary as well.

P.118. Reducing mGlu5 receptors improves survival, symptoms and biological features in SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS) is a late-onset and fatal neurological disease characterized by degeneration of upper and lower motor neurons (MNs). The etiology of ALS remains unknown although glutamate(Glu)-mediated excitotoxicity plays a major role in neurodegeneration. In this scenario, Group I metabotropic Glu receptors (mGluR1, mGluR5), the only excitatory mGluRs, are involved in the regulation of important cellular processes altered in ALS and are over-expressed in different experimental models of the pathology. We have previously shown the presence of abnormal exocytotic release of Glu in the spinal cord of mice expressing high copy number of human SOD1 carrying the G93A point mutation (SOD1G93A). Excessive Glu exocytosis can be triggered by different mechanisms, including activation of presynaptic mGluR1 and mGluR5. As a matter of fact, we have recently demonstrated that genetic knock-down of mGluR1 in SOD1G93A mice had a positive impact on disease progression, life span and biological markers of ALS. Following the same path, here we investigated the role of mGluR5 in ALS. We generated two different SOD1G93A mouse strains with partial (SOD1G93AmGluR5+/-) and total (SOD1G93AmGluR5-/-) receptor reduction. SOD1G93AmGluR5+/- mice showed delayed pathology onset, extension of life span, preservation of spinal MNs and normalization of Glu release induced Group I mGluRs. Unexpectedly, these results were not accompanied by improved motor performances in behavioural tests. When studying SOD1G93AmGluR5-/-, we found a more conspicuous improvement of the pathology onset and of life span. Differently from SOD1G93AmGluR5-/-, these results were also accompanied by significant motor skill amelioration. Overall, our findings demonstrate that mGluR5 down-regulation has a significant impact in-vivo on ALS clinical outcome and, together with previous data on mGluR1, provide a rationale for pharmacological approaches based on the selective block of Group I mGluRs.

P.119. Pharmacological manipulation of Sig1R affects ER-mitochondrial interplay in G93A model of ALS

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Background: Disruptions in ER-mitochondria calcium cycle (ERMCC) are fatal for vulnerable motor neurons. Mitochondria-associated ER membranes (MAMs) are functional domains between both organelles involved in fine Ca2+ exchange. MAMs are enriched with Sig1R, an inter-organelle signaling modulator. Pharmacological manipulation of SigR1 was neuroprotective in hSOD1G93A and wobbler mice. Therefore, SigR1 modulation may provide a treatment option in ALS. Objectives: The aims of this study in the presence and absence of mutated hSOD1 was: to elucidate distribution and activity of SigR1; to determine pharmacological effects of SigR1 agonists on protein expression and its impact on ERMCC in motor and non-motor neurons. Methods: Expression of Sig1R are examined by immunocytochemistry in motor neurons co-cultures and by Western blot in NSC34 cells. Cytosolic Ca2+ dynamics were analyzed by single cell live calcium imaging using fura 2-AM. The cells were repetitively stimulated with kainate and bradykinin in the presence and absence of Sig1R agonists SA4503 and PRE-084. Results: Sig1R accumulations were observed in nontg and hSOD1G93A motor neurons with significantly incrased immunostaining in hSOD1G93A. PRE-084 and SA4503 significantly increased Sig1R signal in nontg

motor neurons. Oposite effect was observed in hSOD1G93A motor neurons. Presence of agonists and thapsigargin increased Sig1R expression in G93A and hSOD1WT NSC34 cells. SA4503 improved cytosolic Ca2+ clearance in nontg and hSOD1G93A neurons when stimulated with kainate or bradykinin. This effect was not seen when bradykinin was applied in presence of PRE-084. Still, in the kainate experiment PRE-084 improved cytosolic Ca2+ clearance in nontg motor neurons. Conclusion: Activation of Sig1R modulates ERMCC, but it addresses different roles of the receptor in nontg and hSOD1G93A neurons. Agonists may change oligomeric state of SigR1 further influencing its chaperoning and Ca2+ signaling modulation. Stabilizing ER-mitochondria interplay by activation of SigR1 could provide new treatment options in genetically different ALS forms.

P.120. Neuroregeneration by targeting the TGF-β system?

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Introduction Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder with no effective treatment so far. The current molecular genetic campaign is increasingly elucidating the molecular pathogenesis of this fatal disease. Previous studies demonstrate high concentrations of Transforming Growth Factor-Î² (TGF-Î²) in Cerebrospinal Fluid (CSF) of several neurodegenerative disorders. High levels of circulating TGF-Î² are known to promote stem cell quiescence leading to the inhibition of adult neurogenesis. Thus, local reduction of TGF-Î² signaling might promote neuronal regeneration. Results Treatment with TGF-Î²1 leads to cell cycle arrest of immortalized neuronal precursor cells in vitro (ReNcell CX cells, Millipore). Subsequently, gymnotic transfer of TGF-Î²RII specific antisense-oligonucleotides (ASOs) reversed the TGF-Î²1 mediated cell cycle arrest. This specific target downregulation resulted in an inhibited TGF-Î²-signaling and consequently reactivation of neuronal precursor cell proliferation, maintaining neuronal differentiation. Methods Data were obtained by quantitative real-time RT-PCR, immunoblotting, immunocytochemistry and cell counting. Conclusion Targeting the TGF-Î²-system by TGF-Î²RII-specific ASO leads to neuroregeneration of neuronal precursor cell proliferation.

P.121. Macrophage Migration Inhibitory Factor as a Modifier of Mutant SOD1 Toxicity in a Mouse Model of ALS

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Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the loss of upper and lower motor neurons in the brain and spinal cord. Transgenic mice that over-express mutant SOD1 develop paralysis and accumulate misfolded SOD1 onto the cytoplasmic faces of intracellular organelles, including mitochondria and endoplasmic reticulum (ER). Recently, macrophage migration inhibitory factor (MIF) was shown to directly inhibit

mutant SOD1 misfolding and binding to intracellular membranes. To test the role of endogenous MIF in modulating SOD1 misfolding in vivo, MIF deficient mice were bred to mice expressing the dismutase inactive mutant SOD1G85R. Completely eliminating endogenous MIF accelerated disease onset and late disease progression and shortened lifespan of mutant SOD1 mice. Accumulation of misfolded SOD1 and association with mitochondria and ER membranes was significantly higher in the spinal cord of SOD1G85R-MIF-/- compared to their SOD1G85R-MIF+/+ littermates. Moreover, the levels of sedimentable insoluble SOD1 aggregates were higher in the spinal cord of these mice. Our findings indicate that MIF plays a significant role in SOD1 folding and misfolding mechanisms in vivo. These results have implications regarding the therapeutic potential role of upregulation of MIF in modulating the specific accumulation of misfolded SOD1.

P.122. MHCI deficiency accelerates muscle denervation in mouse models of amyotrophic lateral sclerosis

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Growing evidence suggest a prominent role of the immune system in the pathogenesis and progression of Amyotrophic Lateral Sclerosis (ALS), the most common and fatal adult-onset motor neuron disease. We recently found that two SOD1G93A mouse models of amyotrophic lateral sclerosis, with different background, exhibited a remarkable difference in disease onset and progression, despite a similar loss of spinal motor neurons. We also noted that the motor neurons of ALS mouse models displaying a less aggressive disease course (C57 SOD1G93A), compared to animals displaying a faster disease progression (129Sv SOD1G93A), up-regulate the immunoproteasome subunit LMP7 and over-expressed the presentation of major histocompatibility complex class I (MHCI), particularly in the peripheral axons during the disease course. Here, we investigated thoroughly the role of MHCI at peripheral level, in particular on the adaptive immune cell recruitment and Schwann cell response in the motor axons of the two SOD1G93A mouse strains and in SOD1G93A mice lacking MHCI signaling (Î²2-microglobulin knock-out mice). We demonstrate that at sciatic nerve level the prominent infiltration of CD8+ T lymphocytes and macrophages, as well an increased response to stress of Schwann cells, contributes to slow down the muscle denervation. By contrast, in absence of MHCI activation, the damaged motor neurons undergo a rapid peripheral nerve degradation and massive muscle denervation determining a faster disease progression. These data unequivocally demonstrate that the preservation of the peripheral nervous system, though a protective response mediated by MHCI, has a positive impact on disease course. This work is supported by the "Thierry Latran" Foundation, the MND Association, the Euro-MOTOR project and the "Amici del Mario Negri" Association.

P.123. Intraspinal injection of human mesenchymal stromal cells in SOD1G93A ALS mice

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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 and appear therefore suitable to create a neuroprotective microenvironment. They can easily and safely be isolated from human bone marrow and are therefore promising candidates for further preclinical and clinical evaluation. Human bone marrow derived mesenchymal stromal cells (hMSCs) are isolated by a previously established GMP-conform protocol. 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 1Âµl as described) , or saline as vehicle control before symptom onset (day 40). 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 rotorod and footprint analysis. Preliminary results of our in vivo studies reveal a significant effect of hMSC injections on general condition, as well as a trend towards increased survival, decreased weight loss and improvement in motor performance. Ongoing studies contain histological analyses of spinal cord tissue and further animal studies assessing different administration routes/ intervals (intrathecal vs. intraspinal, single vs. repeated injections) to assess whether this can improve the protective effect.

P.124. Interleukin-6 blockade improves inflammatory but not metabolic condition in ALS

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Background In Amyotrophic Lateral Sclerosis (ALS), motor neuron degeneration occurs concomitantly with systemic metabolic impairment and neuroinflammation. Interleukin-6 regulates both energy metabolism and inflammation, including complex signalling networks at the-whole-organism level. In this study, we aimed to determine the relevance of the interleukin-6 pathway blockade in ALS. We analysed metabolomics profile and immunologic status of SOD1*G93A transgenic mice treated with a murine analogue of tocilizumab i.e. MR16-1. Methods We used SOD1*G93A (mSOD1) transgenic mice (n=22) and wild-type littermates (n=24), with half of the mice treated by MR16-1 (20 mg/kg, twice/week,10 weeks). We evaluated the metabolomics profile of muscle and cerebral cortex at 20 weeks and plasma samples collected during 20 weeks. We also analysed the clinical (body weight, rotarod performance) and the immunological effects (cytokines, regulatory T cells count in blood) of MR16-1. We compared the evolution of metabolic and immunological profiles between 1) untreated mSOD1 and WT mice and 2) treated mice. We confirmed plasma metabolomics findings in ALS patients. Results We observed a correct discrimination between untreated mSOD1 and WT mice from tissues metabolome ($p<0.001$). These alterations involved free carnitine, saturated fatty acids, and the arginine and proline metabolism pathway, that has been confirmed in ALS patients. We revealed a mild metabolic effect of MR16-1, but a significant increase of regulatory T cells count ($p=0.0268$) and a decrease of CXC ligand-1 concentration ($p=0.0479$). MR16-1 treatment deteriorated clinical outcome, speeding up onset of weight loss ($p = 0.0041$) and decreasing body weight ($p<0.05$). Conclusion Our study shows strong metabolomics alterations in tissues of mSOD1 mice. Interleukin-6 blockade had harmful effects on body weight, in spite of a moderated anti-inflammatory effect. This fundamental result shows that interleukin-6 pathway is relevant in ALS pathophysiology, and this treatment should be optimized for the elaboration of future trials targeting this pathway.

P.125. Impaired exercise tolerance in ALS is related to reduced oxidative metabolism of skeletal muscles

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ALS patients exhibit a pattern of reduced exercise capacity since the earliest phases of the disease but this phenomenon has not yet been fully characterized in terms of the underlying governing dynamics, and specifically in terms of muscle oxidative metabolism. This information is conceivably critical because external validity of exercise trials in ALS is often weak due to the important phenotypic heterogeneity of the disease itself, implying a strong need for standardized solid measures. Aim of this work consisted in exploring the individual exercise tolerance (VO2peak) in ALS patients, especially focusing on the concurrent non-invasive evaluation of oxidative metabolism at skeletal muscle level. 30 ALS patients were compared to 11 age-comparable healthy controls (CTRL). VO2peak was assessed by an incremental exercise test on a cycloergometer, while vastus lateralis muscle O2 extraction capacity by near infrared spectroscopy (NIRS). Lung function was explored in ortho- and clinostatism in resting conditions by spirometry and diffusion lung capacity. ALS patients displayed on average 50% lower VO2peak values vs CTRL, paralleled by significantly lower muscle oxygen extraction capacity (on average 60%). A correlation between these variables was found in ALS patients but no in CTRL; furthermore, ALSFRS-R scores correlated with both paraemters. Spirometric indexes were significantly reduced in clinostatism in ALS patients and significantly related to clinical scores; no correlations with lung diffusion capacity values were shown. In conclusion, impaired skeletal muscle O2 extraction during exercise (including respiratory muscles) is an important determinant of exercise intolerance in ALS patients. A functional evaluation of oxidative metabolism with not invasive methods is a major goal in a multidisciplinary approach aimed at designing tailored exercise interventions for preserving exercise tolerance in ALS patients.

P.126. Identification and characterization of specific nanobodies against the EphA4 receptor

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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. This process leads to progressive muscle wasting in patients. ALS is characterized by considerable genetic heterogeneity since mutations in more than 10 different genes (eg SOD1, FUS, TDP, C9ORF72) are known to cause the hereditary form of ALS. Similarly, heterogeneity is observed in the clinical presentation. This

indicates that there are factors that modify the phenotypic expression of the disease. The tyrosine kinase receptor EphA4 was recently reported to be a modifier of ALS. Genetic and pharmacological inhibition of EphA4 by receptor antagonism was reported to rescue the aberrant motor neuron phenotype in a zebrafish model of ALS. These interventions also increased survival in ALS rodent models. In ALS patients an inverse correlation was found between EphA4 expression and disease onset. Nanobodies are small antigen-binding fragments derived from Camelid heavy-chain antibodies that are devoid of light chains. They have advantages over classical monoclonal antibodies, such as small size, high stability, solubility and low immunogenicity. Here, we aimed to raise specific nanobodies against the EphA4 receptor in order to investigate the therapeutic potential of EphA4 inhibition for ALS. From 15 nanobodies that were generated, we obtained two nanobodies that showed high specificity and low-nanomolar affinity for EphA4. In addition, they completely inhibited the binding of all ephrin ligands to EphA4 receptor. Finally, in U2OS cells both nanobodies inhibited ephrin-induced EphA4 phosphorylation. In summary these results demonstrate a capacity of nanobodies to target EphA4 with high specificity. Future experiments will assess the therapeutic potential of these nanobodies.

P.127. IMPACT OF IFNγ ON NEUROTOXICITY AND ER-MITOCHONDRIA COUPLING CYCLE IN ALS

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Background: Perturbation of the Endoplasmic Reticulum-Mitochondria-Coupling Cycle (ERMCC) appears to be a hallmark of ALS. On the other hand, interferon-gamma, a proinflammatory cytokine, directly triggers calcium transients. In neurons, it induces neurotoxicity in-vitro by forming a calcium-permeable neuron-specific receptor complex4 of IFNGR and GluR1. IFN-gamma control of ERMCC could provide an important direct molecular link between neuroinflammation and neurodegeneration and pave way to therapeutic targets. Objectives: We aim to investigate (i) the distribution and (ii) expression level of IFNGR and AMPAR (iii) effects of IFN-gamma with and without kainate induced excitotoxicity on (iv) neuronal survival and (v) on ERMCC. Methods: Motor neuron co-cultures from E13 mouse ventral spinal cords with (TG) or without (NT) overexpression of mutant hSOD1G93A were used for immunofluorescent staining. Neuronal cell survival was assessed after IFN-gamma treatment with or without kainate for 24 hours. Results: Basal immunofluorescence studies show similar levels of IFNGR and AMPAR distributed across the membranes in both NT and TG motor neurons. Toxicity tests for neuronal survival show that TG neurons appear to be more tolerant to the combined insult from IFN-gamma and kainate unlike the WT neurons. Also, they appear to show a better rescue response when IFNGR is blocked. Further tests will be run to confirm the preliminary findings. Conclusion: Interferon-gamma, an important regulator of neuroinflammation, enhances calcium influx in the neurons. But, surprisingly, hSOD1G93A neurons appear to show lesser susceptibility to neurotoxicity induced by the cytokine. Further investigation is required to understand this observation better. Acknowledgment:

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P.128. Extra virgin olive oil intake ameliorates reticulum stress and muscle damage in SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease associated with mutations in antioxidant enzyme Cu/Zn-superoxide dismutase 1. Albeit there is no treatment for this disease, new insights related to an exacerbated lipid metabolism have been reported. In connection with the hypermetabolic lipid status, the hypothesis whether nature of dietary fat might delay the progression of the disease was tested in transgenic SOD1G93A mice. For this purpose, SOD1G93A mice were assigned randomly to one of the following three experimental groups: (1) a standard chow diet (control, n=21), (2) a chow diet enriched with 20% (w/w) extra virgin olive oil (EVOO, n=22) and (3) a chow diet containing 20% palm oil (palm, n=20). They received the diets for 8 weeks and the progression of the disease was assessed. On the standard chow diet, average plasma cholesterol levels were lower than those mice receiving the high-fat diets. Mice fed an EVOO diet showed a significant higher survival and better motor performance than control mice. EVOO group mice survived longer and showed better motor performance and larger muscle fiber area than animals receiving palm. Moreover, the EVOO-enriched diet improved the muscle status as shown by expression of myogenic factors (Myod1 and Myog) and autophagy markers (LC3 and Beclin1), as well as diminished endoplasmic reticulum (ER) stress through decreasing Atf6 and Grp78. Our findings demonstrate that EVOO may be effective in increasing survival rate, improving motor coordination together with a potential amelioration of ER stress, autophagy and muscle damage. Consequently, both ER and mitochondria are important contributors to the muscle cell fitness in ALS. This is in agreement with the proposed network connecting the sarcomere integrity to mitochondrial oxidative metabolism and the important role of skeletal muscle as a primary target of muscle atrophy and of ALS toxicity.

P.129. Effect of CDNF administration in SOD1-G93A mouse model of Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder affecting motor neurons (MNs) in the ventral horn of spinal cord, brainstem and motor cortex. Patients usually die within 3-5 years of symptoms onset and to date neither cure, nor effective therapy is available. The aim of this study was to evaluate the effect of novel cerebral dopamine neurotrophic factor (CDNF) administration in SOD1-G93A mouse model of ALS. Neurotrophic factors (NTFs) are known to promote survival of MNs in vitro and in vivo; in particular CDNF is highly expressed in muscle, spreads better than other NTFs in brain tissue and itÂ´s crucially involved in the regulation of ER stress, which plays an important role in in the pathophysiology of ALS. Single intracerebroventricular injection of human recombinant CDNF can significantly postpone the development of symptoms and increase lifespan in mice, as well ameliorate motor function as assessed by different motor tests. Immunohistochemistry analyses of post-mortem tissues show that CDNF administration can prevent death of MNs compared to vehicle treated controls. Furthermore, CDNF can preserve neuromuscular junctions (NMJs) innervation and integrity as determined with staining of pre- and post-synaptic terminal in the gastrocnemius muscle. We conclude that CDNF seems to have a strong protective effect in SOD1-G93A mouse model of ALS, promoting survival of MNs and preservation of NMJs, thus resulting in improved motor coordination and mice survival. On the base of our encouraging data, CDNF holds promise as a therapeutic candidate for the treatment of ALS.

P.130. Dysregulation of ROCK&ERK; in ALS: Combinatorial ROCK-ERK treatment 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 now investigated 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 changes in the crosstalk of the two pathways, our strategy now is to administer a combinatorial ROCK-ERK inhibitory treatment. In human ALS tissue we could not show any changes at the RNA-level by qPCR, but activation of cofilin at the protein level was found by Western blot. Our results indicate hyper-phosphorylation of cofilin in ventral spinal cord in ALS. In mice we could observe increased mRNA expression of ROCK1/2 and profilin1. At the protein level, phospho-cofilin and phospho-ERK was significantly increased in transgenic animals. We could further show enrichment of cofilin becomes at denervated neuromuscular junctions (NMJ) and abnormal localization at

presynaptic nerve terminals. Currently, we are testing the therapeutic potential of combinatorial ROCK-ERK inhibitors in mice. Animals are treated daily beginning with 70 days of age by intraperitoneal injection. Animals are monitored by behavioural tests (rotarod, footprint and hanging-wire) weekly and scored daily for general condition. Nerve conduction studies are performed during the symptomatic stage and histological changes (motoneurons/astrocytes) as well as protein- and RNA-levels are analyzed. These studies are still ongoing. Our results demonstrate a pathophysiological role of ROCK/ERK pathways in ALS and warrant for further development of novel therapeutic approaches.

P.131. Developing vertebrate models to highlight the relevance of Nefl and miRNAs in ALS pathogenesis

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A striking number of genes implicated in ALS pathogenesis encode proteins with functions in RNA metabolism. Also, preliminary results suggest that miRNAs are aberrantly expressed in spinal motor neurons in ALS, and that mRNAs encoding neurofilament proteins are a disease relevant target. In order to address the mechanisms involved and identify common therapeutic targets, we decided to take advantage of the zebrafish model, which allows large-scale drug screening and in vivo assessment of biological processes, combined with a wide range of genetic tools : gene over[removed]DNA or RNA injection), knock-down (antisense morpholino injection) or knock out (CRISPR/Cas9), and fluorescent transgenic lines. Therefore, we are developing zebrafish animal models to study in vivo the functions of these RNA-binding proteins, miRNAs and neurofilament proteins, as well as the consequences of their disruption. First of all, we identified and characterized the zebrafish homologues for the low molecular weight neurofilament protein (Nefl) and 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 of the fish). We are currently developing long-term Nefl KO using the CRISPR/Cas9 genome editing tools. We also assess by qRT-PCR and in situ hybridization Nefl expression within ALS zebrafish models. Our collaborators identified a few miRNAs that are aberrantly expressed in ALS patients, and which misregulation affects Nefl expression and aggregation. In parallel, we are performing over-expressions and down regulations of these miRNAs of interest in zebrafish embryos to assess their biological consequences. Establishing these animal models will allow a better understanding of the role of these key actors in ALS pathogenesis and their interactions, and will provide relevant endpoints for future studies to identify novel therapeutics targets for ALS.

P.132. Antisense oligonucleotides approach for the development of Amyotrophic Lateral Sclerosis therapy

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Amyotrophic Lateral Sclerosis (ALS) is a fatal incurable disease caused by motor neuron (MN) degeneration. No effective treatments are presently available. The lack of a clear understanding of ALS causes, in particular in sporadic ALS (sALS), has hampered the searching for a cure. On the other hand, the genetic ALS forms can offer a solid ground for research since, at least in these cases, the etiopathogenetic primum movens is known. In this study, we developed oligonucleotides with morpholino (MOs) chemistry designed to reduce the synthesis of human SOD1 and C9ORF72, the most frequent ALS causative genes. Therapeutic delivery of MOs slowed disease progression, improved neuromuscular function, and increased survival in an in vivo ALS mouse model harboring mutant SOD1G93A. Furthermore, neuropathological evaluation showed an augmented motor neuron and axon numbers and a marked reduction in astrogliosis and microgliosis. To investigate the effectiveness of MO approach in a human model, we treated human fALS (SOD/C9ORF72) induced pluripotent stem cell (iPSC)-derived motor neurons with MOs. After treatment, SOD1 motor neurons displayed increased survival and reduced expression of apoptotic markers, while C9ORF72 cells showed a significant reduction of toxic dipeptides. Overall, our results support the efficacy of MO-mediated therapeutic strategy in ALS models, opening the path for human clinical trials.

P.133. Additive effects of HGF, Artemin and CNTF on ALS-relevant motor neurons isolated by high speed FACS

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Neurotrophic factors regulate the survival of developing motor neurons and hold considerable therapeutic potential in ALS but their combinatorial actions remain poorly understood. Using a novel high speed FACS (fluorescent-activated cell sorting) technique to efficiently isolate pure motor neurons from mouse lumbar spinal cord, we tested the survival effects of 12 neurotrophic factors in 66 double and triple combinations. Our data demonstrate potent strictly additive effects of hepatocyte growth factor (HGF), ciliary neurotrophic factor (CNTF) and artemin (ARTN) on motor neuron survival. We demonstrate that these additive effects involve distinct non-overlapping subsets of motor neurons: HGF specifically promotes the survival of lateral (LMC) motor neurons innervating hindlimb muscles through activation of c-Met kinase. CNTF supports two subsets of medial (MMC) motor neurons innervating

axial muscles through its receptor complex CNTFRalpha/Lifrbeta/gp130. Artemin is a novel neurotrophic factor for preganglionic (PGC) motor neurons through GFRalpha3/Syndecan-3 signaling. These data illustrate the specific action of neurotrophic factors on defined motor neuron subsets and outline a logic for combined neurotrophic factor delivery in ALS and related motor neuron disorders.

P.134. A new AAV-TDP43M337V-based simulation of Amyotrophic Lateral Sclerosis in rats

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Mutations of transactive response DNA binding Protein of 43 kDa (TDP43), including the M337V mutation, are responsible for 1-5 % of familial cases of Amyotrophic Lateral Sclerosis (ALS). In addition, cytoplasmic mislocalization and aggregation of TDP43 is one of the most prominent neuropathological hallmarks of ALS found in 98% of all patients (familial and sporadic). Animal models recapitulating this feature are likely to provide relevant simulations of the human disease. We aimed at developing a new rat model of ALS based on mutant TDP43 pathology which is introduced by adeno-associated viral vectors (AAVs). AAV2/6-CMV-TDP43M337V (AAV-TDP43) and AAV2/6-CMV-GFP (AAV-GFP) (4.1010 viral genomes), used as control, were injected intracerebroventricularly (icv) in male rat neonates at post-natal day one. General locomotion, gait and motor coordination were longitudinally monitored up to 4 month post-injection using locomotor activity boxes, Catwalk analysis and horizontal ladder challenge. Transgene expression and motoneuron loss were histopathologically assessed. Icv injection of AAV-TDP43 in neonates led to hindlimb paresis that translated into attenuated locomotion and rearing, alteration of gait parameters and decreased performance in horizontal ladder walking that worsened with time. Full paralysis of limbs was not observed within the time frame of the study (4 months). In conclusion, our AAV-TDP43M377V-based rat model of ALS developed a progressive and relatively mild ALS-like phenotype, characterized by objective and quantitative motor read-outs, which should prove suitable for future drug testing.

P.135. A PRELIMINARY STUDY OF CHAPERONE-MEDIATED AUTOPHAGY IN ALS LYMPHOMONOCYTES

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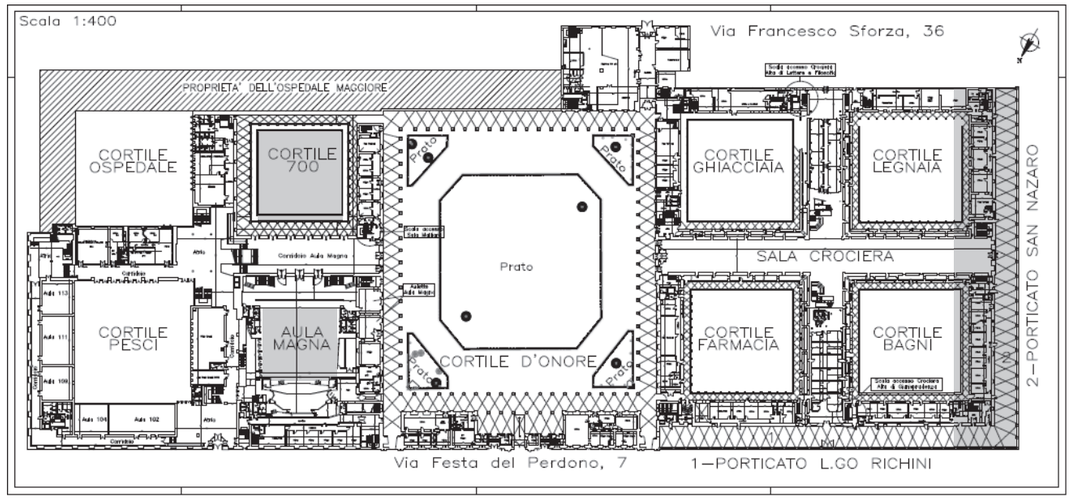
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective degeneration of both upper and lower motor neurons associated to

TDP-43 accumulation. Recent evidences showed that TDP-43 is degraded, at least in part, by the chaperone-mediated autophagy (CMA). In this work, we assessed the expression of the two principal parameters of CMA in peripheral blood mononuclear cells (PBMC) obtained from 30 sporadic ALS patients (sALS), 9 ALS patients carrying mutations in SOD1 gene (SOD1+) and 30 healthy controls (CTRL). Our results showed a significant reduction of hsc70 mRNA and protein levels in sALS and SOD1+ patients with respect to controls, while no changes were observed for lamp2A, the rate limiting protein of CMA. Moreover, to estimate the degradative activity of this pathway, we evaluated the levels of specific and nonspecific substrates: although myocyte enhancer factor 2D (MEF2D) levels were unchanged, we showed increased TDP-43 levels in sALS patients, showing also an increased TDP-43/TDP-35 ratio. Immunohistochemistry analysis, performed on 3 sALS patients and 3 healthy controls, revealed that TDP-43 was aggregated in patients cells outside nuclei. Furthermore, we also investigated parameters related to other degradative mechanisms, reporting a significant reduction of mRNA levels of two macroautophagy-related parameters, SQSTM1/p62 and LC3, in both sALS and SOD1+ patients with respect to controls. Lastly, we assessed mRNA expression of two co-chaperones, BAG1 and BAG3, which regulate trafficking of substrates to ubiquitin-proteasome system and macroautophagy, and we showed decreased levels of BAG1 in both sporadic and familial ALS patients. In conclusion, our results evidenced reduced hsc70 levels with no change in lamp2A, excluding a significant impairment of CMA in PBMC obtained from ALS patients. Anyway, the accumulation of TDP-43 suggests that other degradative pathways could be altered and further investigations are needed in order to explore this hypothesis.

Congress venue on Thursday May 19th and Friday May 20th:
Main Lecture Hall (Aula Magna)
at the University of Milan central campus

Address: University of Milan Medical School
Via Festa del Perdono 7
20122 Milan



Congress venue on Saturday May 21st and Sunday May 22nd:
CASA CARDINALE ILDEFONSO SCHUSTER,

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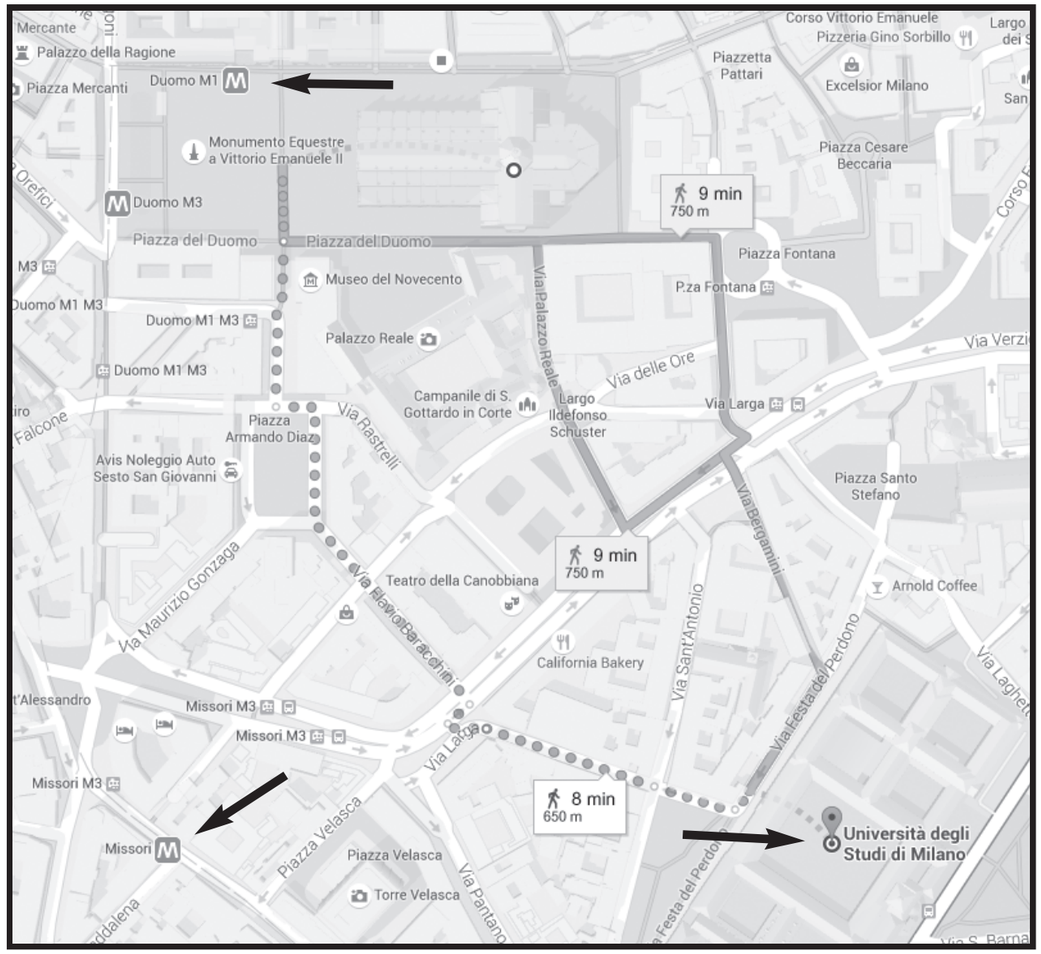
walking distance from the Main Lecture Hall



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NOTE