ENCALS

European Network for the Cure of ALS

PROGRAMME & ABSTRACTS

MAY 21st – 23rd 2015
TRINITY COLLEGE DUBLIN

Trinity College Dublin
The University of Dublin
MND Research

MESSAGE FROM THE ORGANIZERS

Dear participant of the 13th Meeting of ENCALS,

CÉAD MÍLE FÁILTE GO BAILE ATHA CLIATH!

We welcome you to Trinity College Dublin for the 13th meeting of ENCALS.

This meeting is an important forum for the European ALS community - the aim is to encourage younger researchers to present their data, and to meet and interact with more established members of the ALS community.

This year the focus is on Genes and Genomics, Cognition, C9orf72 and Novel Therapeutics, Imaging, TDP: RNA Metabolism and Disease Pathogenesis, Disease Models and Pathogenesis and Novel Biomarkers. We are delighted to have as our guests eminent researchers from both sides of the Atlantic, including Prof. John Landers from University of Massachusetts, Dr. Pat Andres from the Massachusetts General Hospital, Dr. Seward Rutkove from Beth Israel Hospital Boston, Prof. Leonard Petruccelli from the Mayo Clinic, Dr. Francisco Baralle from the International Centre for Genetic Engineering and Biotechnology in Trieste and Dr. Laura Ferraiuolo from the Sheffield Institute for Translational Neuroscience.

As usual for ENCALS, the quality of 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 Idec, Treeway, CCA Clinical Research, Cytokinetics, Fáilte Ireland and administrative support from Trinity College Dublin and Research Motor Neurone.

We thank the members of the scientific committee, Akke Albada (Communications officer) at the University Medical Centre Utrecht for her excellent administrative support of ENCALS and Dominique Plant, who has undertaken most of the local organization.

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

Professor Orla Hardiman
On behalf of the organizing committee
Trinity College Dublin
Ireland

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PROGRAMME OVERVIEW

Thursday, 21st May 2015

12.00 – 13.30	Registration and lunch
13.30 – 14.10	Opening Session: Latran Guest Speaker
14.10 –16.00	Session 1: Genes and Genomics
16.00 –16.30	Coffee
16.30 – 17.30	Session 2: Cognition
17.30 –19.00	Guided Poster Tour with Cheese and Wine Reception
18.00 –19.30	Project MinE Meeting (closed meeting)
19.15	Cytokinetics Investigator Reception (closed meeting)

Friday, 22nd May 2015

09.15 – 11.00	Session 3:C9orf72 and Novel Therapeutics
11.00 –11.30	Coffee
11.30 –12.30	Session 4: Imaging
12.45 –14.15	Lunch and Poster Session
13.00 –14.15	ENCALS Board Meeting (closed meeting)
14.15 –16.00	Session 5: TDP: RNA Metabolism and Disease Pathogenesis
16.00 –16.30	Coffee
16.30 –17.30	Session 6: Models of Disease Pathogenesis (1)
17.30 –19.00	Guided Tour Posters with Wine Reception
20.00	Dinner in the Dining Hall Parliament Square

Saturday, 23rd May 2015

08.30 – 09.00	ENCALS Young Investigator Award
09.00 – 10.45	Session 7: Disease Models and Pathogenesis (2)
10.45 –11.15	Coffee
11.15 – 11.45	Session 8: Novel Biomarkers
12.45 –13.00	ENCALS Meeting Conclusion

Thursday, 21st May

12.00–13.30 Registration and Lunch — Hallway, Ground Floor

13.30–14.35 Opening Session — Stanley Quek Theatre B1
Prof. Van den Berg & Prof. Orla Hardiman

13.35-14.15 Invited Speaker: Latran Foundation: *Prof. Seward Rutkove (USA)*
"EIM as a biomarker in ALS"

14.15 – 15.00 **Oral Session 1: Genes and Genomics**
Chairs: Dr. Russell McLaughlin, Prof. Jan Veldink

14.15-15.00 **State of the Art Speaker:** Prof. John E. Landers (USA)
"Using Next-Generation Sequencing to Dissect ALS Pathogenesis"

15.00 - 15-15 OS1.1: Genetic Overlap between Amyotrophic Lateral Sclerosis and Schizophrenia

R. McLaughlin (Project MinE)

15.15 – 15.30 OS1.2: The Serotonin 2b Receptor prevents Microglia Degeneration and Disease Progression in ALS.

H. el Oussini

15.30 – 15.45 OS1.3: TBK1 Haploinsufficiency causes ALS and FTD

J. Weishaupt

15.45-16.00 OS1.4: Intermediate ATXN2 CAG Expansions predict risk in ALS

W. Sproviero

16.00 - 16.30 **Coffee**

16.30 – 17.30 **Oral Session 2: Cognition**
Chairs: Prof. Sharon Abrahams, Dr. Marwa Elamin

16.30 – 16.45 OS 2.1: The Predictors of Behavioural Change in ALS Patients without Dementia

M. Elamin

16.45 - 17.00 OS2.2: Apathy Profiles in Amyotrophic Lateral Sclerosis, Parkinson's disease and Alzheimer's disease

R. Radakovic

17.00 - 17.15 OS2.3: Cognitive Impairment in ALS assessed with 18F-FDG-PET

U. Manera

17.15 – 17.30 OS2.4 : Cognitive Deficits in Pure ALS: A Data Driven Approach

T. Burke

17.30 - 19.00 **Guided Poster Tour with Cheese and Wine Reception**
B2.72-2.73-2.74 & B2.36-2.37-2.38

18.00 - 19.30 **Project MinE Meeting (closed meeting)**
Stanley Quek Theatre B1

19.15 **Cytokinetics Investigator Reception (closed meeting)**
Alexander Hotel

Friday, 22nd May

09.15 – 11.00 **Oral Session 3: C9orf72 and Novel Therapeutics**

Chairs: Prof. Albert Ludolph, Prof. Pamela Shaw

09.15-10.00 **State of the Art Speaker:** Prof. Leonard Petruccelli (USA) "Mechanisms of Toxicity and Therapeutic approaches for C9FTD/ALS"

10.00- 10.15 OS3.1: Genetic Correction of C9orf72 Repeat Expansion Mutation in ALS/ FTD Patient iPSCs

N. Ababneh

10.15- 10.30 OS3.2: C9orf72 Repeat Expansions produce Distinctive Metabolic Profiles in Human CNS Tissue

G. Valbuena

10.30- 10.45 OS3.3: C9orf72 Epigenetic Modifications in Italian Amyotrophic Lateral Sclerosis Patients

D. Calini

10.45- 11.00 OS3.4: Diaphragmatic Pacing in Motor Neurone Disease: A randomized Controlled Trial (DiPALS)

C. McDermott

11.00-11.30 **Coffee**

11.30-12.30 **Oral Session 4: Imaging**

Chairs: Dr. Esther Verstraete, Dr. Peter Bede

11.30 - 11.45 OS4.1: Simulating Disease spread in Amyotrophic Lateral Sclerosis using a Connectome-Based Model

R. Schmidt

11.45 - 12.00 OS4.2: Physical Disability in ALS is Associated with Functional Connectivity Changes.

J. Machts

12.00 - 12.15 OS4.3: Pathology spreading in ALS: A Single-Center in Vivo DTI-Based Staging Analysis in 289 Patients.

J. Kassubek

12.15 - 12.30 OS4.4: Serum microRNAs in sporadic amyotrophic lateral sclerosis

A. Freischmidt

12.30-14.00 **Lunch and Poster Session**

Knowledge Exchange Level 2

13.00 –14.15 ENCALS Board Meeting (closed meeting)

14.15 – 16.00 **Oral Session 5: TDP: RNA Metabolism and Disease**

Chairs: Dr. Severine Boillee, Prof. Vincenzo Silani

14.15-15.00 **State of the Art Speaker:** Dr. Francisco Baralle (Italy)
"Functional Interactions of TDP 43 and their Role in ALS Pathogenesis"

15.00 – 15.15 OS 5.1: pTDP43 is a Better Correlate of Clinical Phenotype than RNA Foci or GA Dipeptide in C9ORF72 ALS/FTD

J. Scaber

15.15 - 15.30 OS 5.2: Primary Fibroblasts Cultures Reveal TDP-43 Abnormalities in Amyotrophic Lateral Sclerosis Patients.

M. Sabatelli

15.30 – 15.45 OS 5.3: Deciphering the Pathogenic Mechanisms of TCP-43 and FUS Proteinopathies in Novel Ex Vivo Models

E. Hock

15.45 -16.00 Cell-to-Cell Transmission of TDP-43 across Axon Terminals

M. Feiler

16.00 – 16.30 **Coffee**

16.30-17.30 **Oral Session 6: Models of Disease Pathogenesis (1)**

Chairs: Prof. Caterina Bendotti, Dr. Niamh O'Sullivan

16.30 -16.45 Different Requirements for the VCP Co-Factors Npl4 and Ufd1 in Neuronal Function

N. O'Sullivan

16.45 -17.00 Expression of the ALS-causing Variant hSOD1G93A leads to an Impaired Integrity and Altered Regulation

A. Clement

17.00 – 17.15 Characterisation and Motoneuronal Differentiation of Human FUS-ALS Induced Pluripotent Stem Cells

J. Higelin

17.15 -17.30 Macrophage Migration Inhibitory Factor as a Chaperone Inhibiting Accumulation of Misfolded SOD1

A. Israelson

17.30 - 19.00 **Guided Tour Posters with Wine Reception**

B2.72-2.73-2.74 & B2.36-2.37-2.38

20.00 **Dinner in the Dining Hall Parliament Square Trinity College**

Saturday, May 23rd

08.30 - 09.00 ENCALS **YOUNG INVESTIGATIOR AWARD**

09.00 – 10.45 **Oral Session 7: Disease Models and Pathogenesis (2)**
Chairs: Dr. Susanne Petri, Prof. Ludo van den Bosch

09.00 – 09.45 **State of the Art Speaker:** Dr. Laura Ferraiuolo (UK)
"Using *in vitro* Models to Reconstruct Pathogenic Mechanisms in ALS"

09.45-10.00 OS 7.1: Pharmacological Correction of Dysfunctional MNs Differentiated from ALS patient-derived iPSC

M. Naujock

10.00-10.15 OS 7.2: Neuromuscular Junction Formation using hiPSC co-culture system

M. Demestre

10.15-10.30 OS 7.3: Presymptomatic Activation of the PDGF-CC Pathway Accelerates Onset of ALS Neurodegeneration

S. Lewandowski

10.30-10.45 OS7.4: Role of Dynein in the Clearance of Misfolded Proteins Responsible for ALS

R. Cristofani

10.45-11.15 **Coffee**

11.15-12.45 **Oral Session 8: Novel Biomarkers**
Chairs: Prof. Adriano Chio, Dr. Martin Turner

11.15-11.45 **State of the Art Speaker:** Dr. Pat Andres, MS, DPT (USA)
"Use of Quantitative Strength Testing to Measure Disease Progression in ALS"

11.45-12.00 OS8.1: Characterising the Metabolic Profile of ALS: Results from the EuroMotor Study Cohort

A. Siskos

12.00-12.15 OS8.2: Imaging Signatures of ALS

C. Schuster

12.15-12.30 OS8.3: Brain Morphological Changes in Asymptomatic C9orf72 Repeat Expansion Carriers

R. Walhout

12.30-12.45 OS8.4: Abnormal Beta-Band Desynchronisation as a Pre-Symptomatic Biomarker of Motor Network Dysfunction

M. Proudfoot

12.45-13.00 ENCALS Meeting Conclusion

POSTER SESSIONS

Posters will be displayed throughout the meeting. B2.72-2.73-2.74 & B2.36-2.37-2.38

Poster Session I
Thursday 21st May, 17.30-19.00

CLINICAL

P.01: PERCEIVED QUALITY OF LIFE IN AN ALS MULTIDISCIPLINARY CARE UNIT. MULTIDOMAIN ANALYSIS AND FURTHER EVIDENCE OF THE "DISABILITY PARADOX"
Tejado A, , Martinez Y, Begoña A, Perez S, Turon J, Paipa A, Verges E, Povedano M.

ALS Multidisciplinary Care Unit, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Spain.

P.02: EVALUATION OF THE CAREGIVER BURDEN USING THE ZARIT INTERVIEW SCORE IN AN ALS MULTIDISCIPLINARY CARE
Tejado A, Paipa A, Verges E, Martinez Y, Begoña A, Turon J, Povedano M.

ALS Multidisciplinary Care Unit, Hospital Universitari de Bellvitge, L'Hospitalet de llobregat, Spain.

P.03: DEVELOPMENT OF A VALID SET OF GUIDELINES AND QUALITY INDICATORS FOR THE CARE OF PATIENTS WITH ALS
Janssens, A.I.W.A.1; Van Damme, P.2,3; Vanhaecht, K.1,4; Hardiman, O.5,6; Sermeus, W.1

1: Department of Public Health and Primary Care, KU Leuven - University of Leuven, Leuven, Belgium.
2: Department of Neurosciences, Experimental Neurology and Leuven Research Institute for Neuroscience and Disease (LIND), KU Leuven - University of Leuven, Leuven, Belgium.
3: Department of Neurology, University Hospital Leuven, Leuven, Belgium.
4: Department of Quality Management, University Hospitals Leuven, Leuven, Belgium.
5: Academic Unit of Neurology, Trinity College Dublin, Trinity Biomedical Sciences Institute, Dublin, Ireland.
6: Department of Neurology, Beaumont Hospital, Dublin, Ireland.

P.04: A FURTHER RASCH STUDY CONFIRMS THAT ALSFRS-R DOES NOT CONFORM TO FUNDAMENTAL MEASUREMENT REQUIREMENT

Jessica Mandrioli (1), Andrea Giordano (2), Nicola Fini (1), Eleni Georgoulopoulou (1), Antonio Fasano (3), Edoardo Rosi (3), Laura Ferri (3), Franco Franchignoni (4), and ERRALS Group.

1: Department of Neuroscience, St. Agostino-Estense Hospital, Modena, Italy.
2: Unit of Bioengineering, Salvatore Maugeri Foundation, Scientific Institute of Veruno (NO), Italy.
3: University of Modena and Reggio Emilia, Modena, Italy.
4: Unit of Occupational Rehabilitation and Ergonomics, Salvatore Maugeri Foundation, Scientific Institute of Veruno (NO), Italy.

P.05: SCINTIGRAPHIC EVALUATION OF MILD TO MODERATE DYSPHAGIA IN MOTOR NEURON DISEASE

Katarzyna Szacka1, Anna Potulska-Chromik1, Katarzyna Fronczewska-Wieniawska2, SpychaЛa2, Leszek Królicki2, Magdalena Kuzma-Kozakiewicz1,3

1: Department of Neurology, Medical University of Warsaw, Warsaw, Poland.
2: Department of Nuclear Medicine, Medical University of Warsaw, Warsaw, Poland .
3: Neurodegenerative Diseases Research Group, Medical University of Warsaw, Warsaw, Poland.

P.06: QUALIFICATION OF ALS PATIENTS TO LONGITUDINAL HOME REHABILITATION PROGRAM IN POLAND

Jan Sznajder (1,2), Malgorzata Gawel (2,3), Magdalena Kuzma-Kozakiewicz (2,3)

1: Department of Rehabilitation, Józef Pilsudski University of Physical Education in Warsaw, Poland. 2: Department of Neurology, 3Neurodegenerative Diseases Research Group, Medical University of Warsaw, Poland.

P.07: FROM EXTRA-MOTOR TO EXTRA-EXECUTIVE DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS: A STUDY IN GREECE

Foteini Christidi (1), Ioannis Zalonis (1), Michalis Rentzos (1), Panagiotis Ferentinos (2), Efstratios Karavasilis (3), Vasiliki Zouvelou (1), Theodoros Alexakis (1), Nikolaos Karandreas (1), Ioannis Evdokimidis (1)

1: 1st Department of Neurology, Aeginition Hospital, Medical School, National & Kapodistrian University, Athens, GR.
2: 1st Department of Psychiatry, Aeginition Hospital, Medical School, National & Kapodistrian University, Athens, GR.
3: Radiology Research Unit, Medical Imaging Department, National & Kapodistrian University, Athens, GR.

P.08: SEQUENTIAL DECLINE OF EYE MOVEMENT CONTROL IN AMYOTROPHIC LATERAL SCLEROSIS

Martin Gorges1, PhD, Hans-Peter Müller1, PhD, Dorothée Lulé1, PhD, Annemarie Hübers1, MD, Kelly Del Tredici, MD, PhD2, Johannes Brettschneider, MD,1,2 Jürgen Keller1, M.Sc., Albert C. Ludolph1, MD, Jan Kassubek1, MD, Elmar H. Pinkhardt1, MD (Ulm)

1: Department of Neurology, University of Ulm, Ulm, Germany.
2: Section Clinical Neuroanatomy, Department of Neurology, University of Ulm, Ulm, Germany.
3: Center for Neurodegenerative Disease Research (CNDR), University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

P.09: GASTROSTOMY IN PATIENTS WITH MOTOR NEURONE DISEASE: A PROSPECTIVE COHORT STUDY (PROGAS)

Dr. Christopher McDermot (on behalf of the ProGas study Group)
Reader in Neurology, Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK

P.10: WHAT IS THE CAUSE OF WEIGHT LOSS IN AMYOTROPHIC LATERAL SCLEROSIS?

Pauline Vercruysse (1,2), Jerôme Sinniger (1), Hajer El Oussini (1), Jelena Scekic-Zahirovic (1), Dietmar R. Thal (3), 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: Laboratory of Neuropathology – Institute of Pathology, University of Ulm, Germany.

P.11: SUPPORT NEEDS OF INFORMAL CAREGIVERS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS)

MSc, Jessica de Wit 1,PhD, Carin Schröder 1, PhD, Anita Beelen 2, PhD, Leonard van den Berg 3, PhD, Anne Visser-Meily 1

1: Brain Center Rudolf Magnus and Center of Excellence for Rehabilitation Medicine, University Medical Center Utrecht and De Hoogstraat Rehabilitation, Utrecht, The Netherlands.
2: Department of Rehabilition Medicine, Academic Medical Centre, Amsterdam, The Netherlands.
3: Department of Neurology, University Medical Center Utrecht, Utrecht, The Netherlands §Presenting author.

P.12: END-OF-LIFE PRACTICE IN GERMANY COMPARED TO OTHER EUROPEAN COUNTRIES

Helena E.A. Aho-Özhan, M.Sc.1, Jenny Lindner1, Johanna Heimrath, Ph.D.1, Johannes Dorst, M.D.1, Albert C. Ludolph, M.D.1, Dorothée Lulé, Ph.D.1

1: University of Ulm, Department of Neurology, Germany.

P.13: EVALUATION OF EXTRAPYRAMIDAL SIGNS IN AMYOTROPHIC LATERAL SCLEROSIS (EXTRALS STUDY)

Andrea Calvo MD PhD, Francesca Dematteis, MD, Stefania Cammarosano MD, Cristina Moglia MD, Carlo A Artusi, MD, Alberto Romagnolo, MD, Serena Angrisano, MD, Andrea Bernardini, MD, Maurizio Zibetti, MD, Leonardo Lopiano, MD, PhD, Adriano Chiò MD, Mario G Rizzone, MD.

Department of Neuroscience "Rita Levi Montalcini", University of Torino, Torino, Italy

P.14: HEART RATE VARIABILITY IS DECREASED IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

Tino Prell (1), Thomas Ringer (1), Otto W. Witte (1,2), Julian Grosskreutz (1)
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2: Center for Sepsis Control and Care, Jena University Hospital der Friedrich Schiller University Jena, Erlanger Alee 101, 07747 Jena, Germany.

P.15: META-ANALYSIS, AN OPTIMIZED WAY TO EXPLORE SCIENTIFIC LITERATURE, APPLIED TO MOTOR NEURON DISEASES

Mohamed-Mounir El Mendili (1), Pierre-Francois Pradat (1,2), Salma Masmoudi (3,4)

1: Sorbonne University, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d'Imagerie Biomedicale, Paris, France.
2: Departement des Maladies du Systeme Nerveux, Pitie-Salpetriere Hospital, Paris, France.
3: MATRICE project, Sorbonne Univ Paris 1, Paris, France.
4: Institut des Systemes Complexes, Paris, France.

P.16: PILOT ECAS STUDY IN POLISH PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

Natalia Szejko (1), Katarzyna Ciećwierska (1), Beata Pilczuk(1), Magdalena Kuźma-Kozakiewicz (1,2)

1: Department of Neurology.
2: Neurodegenerative Diseases Research Group, Medical University of Warsaw, Warsaw, Poland.

THERAPEUTICS

P.17: TIRASEMTIV (CK-2017357), A FAST SKELETAL MUSCLE TROPONIN ACTIVATOR, FOR THE POTENTIAL TREATMENT FOR AMYOTROPHIC LATERAL SCLEROSIS (ALS)

Shefner JM1, Wolff AA2, Lee J2, Barragan D2, Meng L2, Bian A2, Malik F2, Andrews J2. 1 Barrow Neurological Institute, Pheonix, Arizona, USA 2 Cytokinetics, Inc., South San Francisco, California, USA

P.18: THE ER-MITOCHONDRIA AXIS AS A NEW THERAPEUTIC TARGET FOR ALS: CHARACTERISATION OF NOVEL DRUG SCREENS

Sarah Müller, Nathalie Welsh, Radu Stoica, Kurt De Vos and Chris C.J. Miller, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London.

P.19: FUMARIC ACID ESTERS INDUCE HYPOXIA-INDUCED FACTOR 1A SIGNALING IN OLIGODENDROCYTE PRECURSOR CELLS

Schmauder K. (1), Wiesner D. (1), Bayer H. (1), Barth E. (1), Ludolph A.C. (1), Witting A. (1)

1: Ulm University, Institute of Neurology, Ulm.

P.20: PRECONDITIONING" WITH LATREPIRDINE, AN AMPK ACTIVATOR, DELAYS ALS PROGRESSION IN SOD1G93A MICE

Coughlan KS, Mitchem MR, Hogg MC, Prehn JHM

Departments of Physiology and Medical Physics, RCSI, Dublin 2

P.21: TRANSPLANTATION OF A NEURAL STEM CELL SUBPOPULATION AS CELL-BASED THERAPY FOR ALS

Agnese Ramirez, Monica Bucchia, Chiara Simone, Monica Nizzardo, Federica Rizzo, Sara Dametti, Stefania Corti

For all authors: Dino Ferrari Centre, Department of Neurological Sciences, University of Milan, IRCCS Foundation Ca' Granda Maggiore Hospital Policlinico, Milan, Italy.

P.22: DHA SUPPLEMENTATION EXTENDS SURVIVAL AND REDUCES INFLAMMATION MARKERS IN MALE ALS MICE

Pascual Torres, Daniel Cacabelos, Victòria Ayala, Jordi Boada, Rosanna Cabré, Alba Naudí, Monica Povedano (1), Reinald Pamplona, Manuel Portero-Otín
Department of Experimental Medicine-NUTREN, IRBLLEIDA-University of Lleida, Lleida, Catalonia, Spain.

Hospital Universitari de Bellvitge-IDIBELL, L'Hospitalet de Llobregat, Catalonia, Spain.

P.23: TARGETING C9ORF72 EXPANDED RNA G-QUADRUPLEX BY SMALL MOLECULE LEAD COMPOUNDS

Roberto Simone1, Pietro Fratta1, Rubika Balendra1, Stephen Neidle2, Gary Parkinson2 and Adrian Isaacs1

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EPIDEMIOLOGY

P.24: THE ASSOCIATION BETWEEN NERVOUS SYSTEM DRUGS AND INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS

Fabrizio D'Ovidio, Angelo d'Errico**, Andrea Calvo*, Adriano Chiò* * Department of Neuroscience - University of Turin (Italy) ** Epidemiology Department ASL TO3 – Piedmont Region (Italy)*

P.25: ALS IN SLOVENIA – ANALYSIS OF A PATIENT COHORT AT THE LJUBLJANA ALS CENTRE

Mojca Kirbis[12], Blaz Koritnik[1], Lea Leonardis[1], Leja Dolenc Groselj[1], Polona Klinar[1], Stanka Ristic Kovacic[1], Janez Zidar[1]

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2: Department of Neurology, General Hospital Nova Gorica, Slovenia.

P.26: BAYESIAN MODELLING OF POTENTIAL ASSOCIATION BETWEEN SOIL MINERAL LEVELS AND SMALL AREA SPATIAL RISK

James Rooney (1), Katy Tobin (1), Mark Heverin (1), Alice Vajda (1), Arlene Crampsie (2), Anthony Staines (3), Orla Hardiman (1,4)

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2: School of Geography, Planning & Environmental Policy, University College Dublin.
3: School of Nursing and Human Sciences, Dublin City University, Dublin, Ireland.
4: Department of Neurology, Beaumont Hospital, Beaumont, Dublin 9, Ireland.

P.27: SURVIVAL ANALYSIS OF SOCIAL DEPRIVATION AND OTHER SPATIALLY STRUCTURED FACTORS IN THE IRISH ALS COHORT

James Rooney(1), Tom Burke(1), M Galvin(1), Katy Tobin(1), Mark Heverin(1), Alice Vajda (1), Marwa Elamin (1), Orla Hardiman(1,2)

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P.28: HIGH RESOLUTION BAYESIAN SMOOTHED SPATIAL RISK MAPPING AND CLUSTER ANALYSIS OF ALS IN IRELAND

James Rooney (1), Katy Tobin (1), Mark Heverin (1), Alice Vajda (1), Arlene Crampsie (2), Anthony Staines (3), Orla Hardiman (1,4)

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BIOMARKER

P.29: ALTERATION OF KINESINS EXPRESSION INVOLVED IN BIDIRECTIONAL TRANSPORT IN BLOOD OF PATIENTS WITH MND

Magdalena Kuzma-Kozakiewicz1,3, Beata Kazmierczak 2,3, Agnieszka Chudy2, Beata Gajewska2,3, Anna Baranczyk-Kuzma 2,3

1: Department of Neurology, 2: Department of Biochemistry, 3: Neurodegenerative Diseases Research Group, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland.

P.30: BLOOD-BASED MIRNAS POTENTIAL BIOMARKERS IN MOTOR NEURONE DISEASE

Rachel Waller, Emily Goodall, Mbombe Kazoka, Helen Wollff, Janine Kirby, Pamela Shaw

Sheffield Institute for Translational Neuroscience, Sheffield University, UK

P.31: MONOCYTE SUBTYPES IN ALS

Lisa Zondler(1), Samira Khalaji(2), Kathrin Müller(1), Corinna Bliederhäuser(1), Veselin Grozdanov(1), Wolfgang P. Ruf(1), Axel Freischmidt(1), Patrick Weydt(1), Albert C. Ludolph(1), Kay-E. Gottschalk(2), Karin M. Danzer(1), Jochen H. Weishaupt(1)

1: Ulm University, Neurology Department.
2: Ulm University, Department of Experimental Physics.

P.32: DOES MUNIX METHOD REFLECT CLINICAL DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS?

Malgorzata Gawel MD, PhD, Magdalena Kuzma-Kozakiewicz MD, Ass Prof.
Department of Neurology, Medical University of Warsaw, 1A Banacha street, 02-097 Warsaw, Poland, Neurodegenerative Diseases Research Group, Medical University of Warsaw, Warsaw, Poland

P.33: The ALS Stratification Prize- Using Big Data and Crowdsourcing for Catalyzing Breakthroughs in ALS

Neta Zach, Robert Kueffner, Hagit Alon, Raquel Norel, Thea Norman, Lara Mangarvite, Orla Hardiman, Nazem Atassi, Merit Cudkowicz, Alexander Sherman, Jason Walker, Ervin Sinani, Igor Katsovskiy, David Schoenfeld, Melanie Leitner, Gustavo Stolovitzky

1: Prize4Life, Tel Aviv, Israel.
2: Department of Informatics, Ludwig-Maximilians-University, Munich, Germany.
3: IBM T.J. Watson Research Center, Yorktown Heights, NY, USA.
4: Sage bionetworks, Seattle, WA, USA.
5: Department of Neuroscience, Beaumont Hospital and Trinity College Dublin, Dublin, Ireland.
6: Neurological Clinical Research Institute, Massachusetts General Hospital, Charlestown, MA, USA.
7: MGH Biostatistics Center, Massachusetts General Hospital, Boston, MA, USA.
8: Biogen Idec, Boston, MA, USA.

P.34: THE ROLE OF THE SENSEWEAR DEVICE AND GHRELIN FOR METABOLISM IN AMYOTROPHIC LATERAL SCLEROSIS

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P.35: RETINAL INVOLVEMENT IN ALS: A STUDY WITH OPTICAL COHERENCE TOMOGRAPHY AND DIFFUSION TENSOR IMAGING

Annemarie Hübers1, Kathrin Böhm1, Jens Dreyhaupt2, Hayrettin Tumani1, Jan Kassubek1, Hans-Peter Müller 1, Albert C. Ludolph1, Elmar H. Pinkhardt1
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P.36: POST MORTEM MRI TO DECIPHER CORPUS CALLOSUM INVOLVEMENT IN ALS

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P.37: ELEVATED INFLAMMATORY PARAMETERS IN WOMEN WITH PROGRESSIVE BULBAR PALSY

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P.39: QUANTIFYING SPINAL CORD ATROPHY: A MRI TOOL TO INVESTIGATE THE SELECTIVITY OF MUSCLE WEAKNESS IN SMA

Mohamed-Mounir El Mendili (1), Timothee Lenglet (2,3), Maria del Mar Amador (4), Stephane Lehericy (5,6), Habib Benali (1), Pierre-Francois Pradat (1,7)
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P.40: CALCIUM DISTURBANCE IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH ALS

Jingyu Liu, Tino Prell, Ayse Malci , Vedrana Tadic, Otto W. Witte, Julian Grosskreutz

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P.41: POLYUNSATURATED FATTY ACID COMPOSITION OF BLOOD LIPIDS AS A POTENTIAL BIOMARKER FOR ALS PATIENTS

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P.42: UNRAVELLING THE MOLECULAR MECHANISMS BEHIND CORTICOSPINAL MOTOR NEURON DEGENERATION IN ALS

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POSTER SESSION II
Friday 22nd May, 17.30-19.00

GENETICS

P.43: ASSOCIATION OF GSTP1 POLYMORPHISM WITH GLUTATHIONE S-TRANSFERASE AND PEROXIDASE ACTIVITY IN MND

Beata Gajewska1,2, Beata Kazmierczak1,2, Magdalena Kuzma-Kozakiewicz 2,3, Anna Baranczyk-Kuzma 1,2

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P. 44: TURKISH ALS CASES WITH THE C9ORF72 EXPANSION: A GENOMIC AND METHYLOMIC APPROACH

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P.45: ALS-ASSOCIATED GENES AS BLOOD RNA BIOMARKERS OF SPINOCEREBELLAR ATAXIA TYPE 2

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P.46: A NOVEL SPG4 GENE MUTATION CAUSING A UPPER MOTOR NEURON PHENOTYPE WITH NEURONOPATHY. AN ALS MIMIC

Verges E, Paipa A, Turon J, Lopez-Toledano E, Povedano M. ALS Multidisciplinary Care Unit, Hospital Universitari de Bellvitge and Centre de diagnostic molecular – Idibell.

P.47: ALS PHENOTYPE ACCORDING TO HFE P.HIS63ASP POLYMORPHISM: AN ITALIAN MULTICENTRE STUDY

Umberto Manera, Andrea Calvo, Cristina Moglia, Giuseppe Fuda, Mario Sabatelli, Marcella Zollino, ITALSGEN, Silvana Penco, Christian Lunetta, Gabriele Mora, Stefania Battistini, Jessica Mandrioli, Gabriella Restagno, Maura Brunetti, Marco Barberis, Adriano Chio'

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P.48: THE GERMAN PRE-SYMPTOMATIC ALS STUDY (GPS-ALS) 2015

Patrick Weydt, Melanie Madinger, Christiane Schaldecker, Antje Knehr, Sarah B?hm, Martin Gorges, Jan Kassubek, Jochen Weishaupt, Department of Neurology, Ulm University, Germany, Johannes Prudlo, Department of Neurology, Rostock University, Germany, Peter Andersen, Department of Pharmacology and Clinical Neurosciences, Umea University, Sweden, Albert Ludolph, Department of Neurology, Ulm University, Germany

P.49: GENETIC MONITORING OF FTLD-ALS SPANIARD PATIENTS

Borrego-Hernandez D.1, Juarez-Rufino, A.1, Atencia, G.1, Cordero-VÁzquez, P.1, Martín, M.A.2, Muñoz-Blanco, J.L.3, Galan, L.4, Esteban-Pérez, J.1 and Garcia-Redondo, A.1

P.50: MOLECULAR CHARACTERIZATION OF TUBA4A GENE IN A POPULATION OF ITALIAN PATIENTS AFFECTED BY AMYOTROPHIC LATERAL SCLEROSIS

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P.51: WHOLE-BLOOD GLOBAL DNA METHYLATION; SELECTION OF ALS PATIENTS FOR GENOME-WIDE METHYLATION PROFILING

Hamid Hamzeiy, Ceren Iskender, Nesli Ece Sen, A. Nazli Basak Department of Molecular Biology and Genetics, NDAL, Bogazici University, Istanbul, Turkey

P.52: THE SPECTRUM OF CLINICAL OPINION ON GENETIC TESTING IN ALS
Alice Vajda, Russell L McLaughlin, Owen Thorpe, Ammar Al-Chalabi, Sharon Abrahams, Orla Hardiman, Trinity College Dublin

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P.53: SOD1 MISFOLDING – A POTENTIAL TARGET FOR THERAPEUTIC INTERVENTION IN ALS
Engel Stanislav, Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel

P.54: PRIMARY MOTOR NEURONS EXPRESSING P525L-FUS DISPLAY FUS MISLOCALISATION AND REDUCED SURVIVAL
Louisa Kent, Thomas Vizard, Kevin Talbot, Matthew Thomas, Javier Alegre Abarrategui, Richard Wade-Martins, Kevin Talbot
Affiliation: Oxford University

P.55: HUMAN IPS CELLS ALLEVIATE ALS PROGRESSION AND IMPROVE THE STRUCTURE OF AFFECTED EXTRACELLULAR MATRIX
Serhiy Forostyak 1,2, Jessica Kwok 3, Pavla Jendelova 1,2, Ales Homola 1,2, James Fawcett 3 and Eva Sykova 1,2
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P.56: TDP-43 DISRUPTS ENDOPLASMIC RETICULUM-MITOCHONDRIA ASSOCIATIONS AND CALCIUM HOMEOSTASIS
Radu Stoica, Sebastien Paillusson, Chris Miller Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London

P.57: THE ROLE OF NEUROTROPHIC FACTORS IN EXTRAOCULAR MUSCLE SPARRING IN AMYOTROPHIC LATERAL SCLEROSIS
Vahid M.Harandi

Departments of Integrative Medical Biology, Section for Anatomy, Umeå University, Umeå, Sweden

P.58: PHOSPHORYLATION OF FUS AFFECTS ITS NUCLEAR IMPORT
Simona Darovic1, Sonja Prpar Mihevc1, Vera Župunski2, Maja Šatlekar1, Youn-Bok Lee3, Christopher Shaw3, Boris Rogelj1

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P.59: NEW INSIGHTS ON HEMATOPOIETIC STEM CELLS DIFFERENTIATION IN TRANSGENIC SOD1G93A MICE

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P.60: DIRECT INTERACTION BETWEEN MACROPHAGE MIGRATION INHIBITORY FACTOR AND SOD1

Guy Zoltsman and Adrian Israelson Department of Physiology and Cell Biology, Ben-Gurion University of the Negev, Israel

P.61: SMAD PROTEINS MEDIATE TGFBETA1 EFFECTS IN ALS MUSCLE

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

P.62: BID POSITIVELY REGULATES THE TLR – NF-KAPPAB INFLAMMATORY RESPONSE IN A SOD1G93A ALS MODEL

Sinéad Kinsella, Hans-Georg Koenig, Jochen H. M. Prehn. Dept. of Physiology and Medical Physics, RCSI.

P.63: ALTERED CHLORIDE HOMEOSTASIS IN EMBRYONIC SPINAL SOD1G93A MOTONEURONS

Elodie Martin, William Cazenave, Anne-Emilie ALLAIN, Daniel Cattaert, Pascal Branchereau Univ. Bordeaux, INCIA, UMR 5287, F-33615 Pessac, France. CNRS, INCIA, UMR 5287, F-33615 Pessac, France

P.64: UPREGULATION OF MICRORNA-155 PRECEDES SYMPTOMATIC INFLAMMATION IN THE SPINAL CORD OF TGSOD1G93A MICE

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P.65: SEARCH FOR MIRNA-REGULATED MOLECULAR NETWORKS IN ALS AND PRION DISEASE

Toivonen JM1, Calvo AC (1), Oliván S1, Manzano R (1), Sanz-Rubio D (1), Espejo-Porras F (2), De Lago E (2), Martín-Burriel I (1), Zaragoza P (1), Osta R (1).

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P.66: THE ROLE OF CASPASE 6 IN ALS

Marion C Hogg 1, Mollie R Mitchem 1, Hans-Georg König 1, & Jochen H.M.Prehn 1,2.

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P.67: EVALUATION OF PRION-LIKE TRANSMISSIBILITY IN ALS: INSIGHTS FROM LABEL-FREE PROTEOMIC ANALYSIS

Luciana Pizzatti1, Auderlan Mendonça de Góis2, Thais Andrade de Lima2, Luciana Oliveira2, José Suelton Luiz Costa dos Santos2, José Fabio Santos Leopoldino3, Marli Pernes4, Claudio Heitor Tavares Gress4, José Mauro Braz de Lima4, Regina Helena da Silva5, Alessandra Mussi de Ribeiro5, Eliana Abdelhay6, Leila Chimelli7, José Ronaldo dos Santos2, Deise Maria Furtado de Mendonça2. Departamento de Química, Instituto de Química, Universidade Federal do Rio de Janeiro7; Departamento de Biociências, Universidade Federal de Sergipe2; Hospital Universitário de Sergipe, Universidade Federal de Sergipe3; Instituto de Neurologia Deolindo Couto, Universidade Federal do Rio de Janeiro4; Departamento de Fisiologia, Universidade Federal do Rio Grande do Norte5; Divisão de Laboratórios do CEMO, Instituto Nacional do Câncer6; Instituto Estadual do Cérebro/Rio de Janeiro7.

P.68: EFFECT OF PROTEIN QUALITY CONTROL SYSTEM ON TDP43 ACCUMULATION IN ALS MOTONEURONAL AND MUSCLE MODELS

Maria Elena Cicardi, Valeria Crippa, Rita Galbiati, Marco Meroni, Riccardo Cristofani, Paola Rusmini, Angelo Poletti. Sezione di Biomedicina ed Endocrinologia, Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milano, Italy. Centro InterUniversitario sulle Malattie Neurodegenerative, Università degli Studi di Firenze, Milano, Genova e Roma Tor Vergata, Italy

P.69: GOLGI PATHOLOGY IN SOD1-ALS IS MEDIATED BY STATHMINS 1/2, MICROTUBULES AND GOLGI SNARES

Sarah Bellouze, Gilbert Baillat, Dorothee Buttigieg, Nathalie Cavanne, Catherine Rabouille, Georg Haase Institut de Neurosciences de la Timone, CNRS and Aix-Marseille University, Marseille, France. Hubrecht Institute for Stem Cell Research and Developmental Biology, Utrecht, The Netherlands.

P.70: THE ROLE OF SIGMA-1 RECEPTOR IN THE ER-MITOCHONDRIA CALCIUM CYCLE IN THE G93A MOUSE MODEL OF ALS

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P.71: SOD1 REDUCTION MORPHOLINO-MEDIATED AMELIORATES AMYOTROPHIC LATERAL SCLEROSIS DISEASE PHENOTYPE

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P.72: A ROLE FOR ER SHAPING PROTEINS IN MITOCHONDRIAL NETWORK ORGANIZATION

Philippa Fowler and Dr Niamh C. O'Sullivan

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DISEASE MODELS

P.73: TRANSCRIPTOME OF MOTOR NEURON SOMAS AND AXONS DERIVED FROM EMBRYONIC STEM CELLS USING MICROFLUIDICS

Julio Cesar Aguila Benitez, PhD Department of Neuroscience, Karolinska Institutet Sweden

P.74: THE SPHINGOSINE 1-PHOSPHATE RECEPTOR 1 (S1P1) AGONIST FTY720 (FINGOLIMOD) IN A RAT MODEL OF ALS

Michel Alexander Steiner, Hugues Lecourt, Francois Jenck Department of CNS Pharmacology-Neurobiology Actelion Pharmaceuticals Ltd Gewerbestrasse 16 4123 Allschwil Switzerland

P.75: DEREGULATED GENE/PROTEIN EXPRESSION IN ASTROCYTES FROM MSOD1 MICE PUPS MIMIC THE SYMPTOMATIC STAGE

Cátia Gomes1, Carolina Cunha1, Filipe Nascimento2,3, Ana Sebastião2,3, Ana Rita Vaz1,4, Dora Brites1,4

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P.76: MODELING MOTOR NEURON DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS USING EMBRYONIC STEM CELLS

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P.77: PGC-1ALPHA EXPRESSION AND FUNCTION IN MOUSE MODELS OF AMYOTROPHIC LATERAL SCLEROSIS

Hanna Bayer(1), Kerstin Lang(1), Johannes Hanselmann(1), Irma Merdian(1), Luc Dupuis(2), Patrick Weydt(1), Anke Witting(1)

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P.78: DIFFERENTIAL EXPRESSION OF NEUROTROPHIC FACTORS IN SPINAL CORD AND MUSCLE OF MUTANT SOD1 MICE

Kefalakes E., Thau-Habermann N., Jekel M., Grothe C., Petri S. Ekaterini Kefalakes, Department of Neurology, Hannover Medical School Marco Jekel, Department of Neurology, Hannover Medical School Nadine Thau-Habermann, Department of Neurology, Hannover Medical School Claudia Grothe, Department of Neuroanatomy, Hannover Medical School Susanne Petri, Department of Neurology, Hannover Medical School

ABSTRACTS

OS1.1: GENETIC OVERLAP BETWEEN AMYOTROPHIC LATERAL SCLEROSIS AND SCHIZOPHRENIA

R. McLaughlin / for Project MinE Consortium

Both sporadic and familial amyotrophic lateral sclerosis (ALS) are heritable, yet only a small proportion of cases can currently be explained by known genetic mutations. The largest genome-wide association studies (GWAS) to date have only confirmed the association of a small number of loci with ALS risk, leaving the remainder of the heritability of ALS weakly identifiable using common genetic variation. This contrasts with other traits such as schizophrenia, for which 108 loci have recently been implicated in a large GWAS, and for which common variation can explain as much as 23% of the variance in liability for disease. The availability of summary statistics for such GWAS provides the opportunity to conduct cross-trait studies of pleiotropy and genetic correlation, allowing potential to discover novel disease mechanisms and genetic risk factors. Prompted by the recent observation that pedigrees of ALS patients are enriched for neuropsychiatric disease, we used GWAS summary statistics to investigate the overlap between schizophrenia and ALS. Using linkage disequilibrium score regression, we replicate estimates for the SNP-based heritability of ALS (8.6%) and we estimate the genetic correlation between ALS and schizophrenia captured by common genetic variation to be 30%. This is supported by quantile-quantile plots for ALS summary statistics conditioned on schizophrenia p-values showing increased inflation in ALS statistics with increasing schizophrenia p-value threshold. The pleiotropic signal is further evidenced by ALS polygenic risk scores for schizophrenia-associated SNPs, for which 3% of the variance is explained by case-control status. These findings have profound implications for our understanding of the underlying biology of ALS as well as its classification and potential treatment.

OS1.2: THE SEROTONIN 2B RECEPTOR PREVENTS MICROGLIA DEGENERATION AND DISEASE PROGRESSION IN ALS

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Amyotrophic lateral sclerosis is associated with the degeneration of non-motorneuronal populations, in particular brainstem serotonergic neurons. The degeneration of serotonin neurons was associated with a late-onset, massive upregulation of the gene encoding serotonin 2B receptor (Htr2b) in spinal cord of SOD1 (G86R) mice. Our objective here was to investigate the role of HTR2B in ALS, in both mutant SOD1 mice and patients. Knocking out Htr2b drastically shortened survival of SOD1 (G86R) mice, while not affecting disease onset. Consistently, ablation of Htr2b exacerbated motor neuron atrophy, as well as aggregation of p62 positive vesicles at end-stage. The upregulation of Htr2b was restricted to microglia. Consistent with a modulatory role on microglial function, loss of Htr2b led to a strong increase in degenerating microglia while decreasing globally the expression of microglia-restricted, pro-inflammatory and anti-inflammatory genes. In patients, we identified several single nucleotide polymorphisms (SNPs) in the human HTR2B gene that were associated with survival in ALS patients in a cohort of Dutch patients. Further analysis is ongoing to determine whether these SNPs are affecting HTR2B expression and microglial survival in patients. In all, our findings identify HTR2B as a master gene controlling both microglial health and function, and disease progression during ALS.

OS1.3: TBK1 HAPLOINSUFFICIENCY CAUSES ALS AND FTD *Axel Freischmidt(1) Thomas Wieland(2,*), Benjamin Richter(3,*), Wolfgang Ruf(1,*), Veronique Schäffer(3,*), Kathrin Müller(1), Nicolai Marroquin(1,17), Frida Nordin(4), Annemarie Hübers(1), Patrick Weydt(1), Susana Pinto(5), Rayomond Press(6), Stéphanie Millecamps(7), Nicolas Molko(8), Emilien Bernard(9), Claude Desnuelle(10), Marie-Hélène Soriani(10), Johannes Dorst(1), Elisabeth Graf(2), Ulrika Nordström(4), Marisa Feiler(1), Stefan Putz(11), Tobias M. Boeckers(11), Thomas Meyer(12), Andrea S. Winkler(13), Juliane Winkelman(13), Mamede de Carvalho(14), Dietmar R. Thal(15), Markus Otto(1), Thomas Brännström(16), Alexander E. Volk(17,18), Pourya Sarvari(19), Didier Y.R. Stainier(19), Petri Kursula(20), Karin M. Danzer(1), Peter Lichtner(2), Ivan Dikic(3), Thomas Meitinger(2,21,22), Albert C. Ludolph(1), Tim M. Strom(2,21,*), Peter M. Andersen(1,4,*), Jochen H. Weishaupt(1,*)*

Amyotrophic lateral sclerosis (ALS) is a genetically heterogeneous neurodegenerative disorder hallmarked by adult-onset loss of motor neurons. We performed exome sequencing of 252 familial cases and 827 control individuals. Gene-based rare variant analysis identified an exome-wide significant enrichment of loss-of-function (LoF) mutations in the gene TANK-binding kinase 1 (TBK1) in fALS patients. No enrichment of LoF mutations was observed in a targeted mutation screen of 1,010 sporadic cases and 650 additional control individuals. Linkage analysis in 4 families gave an aggregate LOD score of 4.6. In vitro experiments confirmed loss of expression of TBK1 LoF mutant alleles, or loss of interaction of the c-terminal TBK1 coiled-coil domain (CCD2) with the TBK1 adaptor protein optineurin, an established ALS gene. We conclude that haploinsufficiency of TBK1 causes ALS and fronto-temporal dementia (FTD).

OS1.4: INTERMEDIATE ATXN2 CAG EXPANSIONS DO PREDICT RISK IN ALS: SYSTEMATIC REVIEW AND META-ANALYSIS *William Sproviero1, Aleksey Shatunov1, Daniel Stahl1, Maryiam Shoe2, Wouter van Rheenen3, Ashley R Jones1, Peter M. Andersen4, Christine van Broeckhoven5, Luisa F. Confortí6, Philip Van Damme7, Hussein Daoud8, Matthew D. Figley9, Monica Forzan10, Cinzia Gellera11, Aaron D. Gitler12, Edor Kabashi13, Vincenzo La Bella14, Serena Lattante15, Vincent Meininger16, Stephanie Millecamps15, Tim Van Langenhove5,*

Yin Chung Lee17, Rosa Rademakers18, Wim Robberecht7, Guy Rouleau19, Owen A. Ross20, Francois Salachas21, Bradley N Smith1, Gianni Sorarù10, Rossella Spataro14, Bing-Wen Soong17, Claire Troakes22, Jan H. Veldink3, Leonard H. van den Berg3, Christopher E. Shaw1, John Hardy2, John F Powell1, Ammar Al-Chalabi1

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Intermediate expansions of a trinucleotide CAG repeat in the ATXN2 gene are a cause of spinocerebellar ataxia type 2 (SCA2) and of amyotrophic lateral sclerosis (ALS). The minimum repeat size conferring risk of ALS is not clear. We systematically searched PubMed for case-control studies published between 1 Aug 2010 and 30 Nov 2014 which reported association between ATXN2 trinucleotide repeat alleles and ALS. Where required, raw data and missing information were obtained directly from authors. Two novel case-control studies were also included, one from the UK and one from Netherlands. We performed a meta-analysis of the relative risk conferred by ATXN2 trinucleotide repeat alleles of size 24 to 34. Relative risk estimates were calculated considering the frequency of each allele compared to pooled frequencies of alleles with 23 repeats or fewer. The UK samples were of 1474 ALS cases and 567 controls; the Dutch samples were of 1328 ALS cases and 691 controls. They were added to data from 13 studies at low risk of bias comprising 10888 ALS individuals and 15463 controls. There was no association of ALS with alleles smaller than 28 repeats in size. There was a significant increase in the risk of ALS for alleles of 29 to 33 repeats: allele 29, 1.68 (95% CI, 1.11-2.54); allele 30, 2.58 (95% CI, 1.21-5.52); allele 31, 2.96 (95% CI 1.73-5.05); allele 32, 8.37 (95% CI, 4.02-17.43), and allele 33, 4.73 (95% CI, 1.92-11.63). ATXN2 alleles of between 29 and 33 repeats are associated with ALS. The risk of ALS increases exponentially with repeat length for alleles of between 29 and 32 repeats, the first time the allele size of a trinucleotide repeat disease has been shown to correlate with risk rather than age of onset.

OS 2.1: THE PREDICTORS OF BEHAVIOURAL CHANGE IN ALS PATIENTS WITHOUT DEMENTIA

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Background: It is now well recognised that ALS can be associated with behavioural impairment even in the absence of frank co-morbid dementia. The clinical and cognitive correlates of these behavioural changes are yet to be established.
Objectives: We aimed (1) to document the frequency of behavioural changes in ALS patients without dementia and (2) to investigate the clinical and cognitive correlates of these changes.
Methods: A cohort of Irish and Scottish ALS patients (n=125 and n=85 respectively) with no evidence of dementia were screened for cognitive and behavioural impairment using the Edinburgh Cognitive and Behavioural Assessment Scale (ECAS). Normative data was generated using age, sex, and education matched healthy control (71 Scottish controls and 40 Irish controls). Disease severity was estimated using the ALSFRS-R and respiratory function was measured using the SNIP and FVC. Behavioural impairment was defined as the presence of at least one behavioural change on informant-based behavioural screen.
Results: Behavioural impairment was documented in 36.6% of ALS patients compared 3.3% of controls ($p<0.0001$).
The most commonly reported behavioural changes in ALS were apathy (20%) and loss of sympathy (18.9%). Other reported changes included perseveration and altered dietary preferences/binge eating (14.3% each) and disinhibition (9.5%). Psychotic symptoms, such as hallucination and delusions, were less common (6.0%).
Apathy was associated with older age ($p=0.003$). No significant associations were observed between reported behavioural changes and disease severity or respiratory function.

Impairment on tasks on tasks of language and social cognition, rather than those of executive functions, predicted higher risk of behavioural impairment particularly disihibition (p=0.008 and p=0.024 respectively).
Conclusion: The ECAS provides rich and specific information regarding behaviour in ALS. Behavioural impairment in ALS is predicted by performance on tasks of social cognition and language and not executive dysfunction, indicating a separate subtype of ALS patients.

OS2.2: APATHY PROFILES IN AMYOTROPHIC LATERAL SCLEROSIS, PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE *Ratko Radakovic1234, John M. Starr3, Richard Davenport2 and Sharon Abrahams124*

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Background: Apathy is defined as a disorder of diminished motivation and is the most prevalent neuropsychiatric symptom in ALS. Although apathy is commonly measured as a unidimensional concept by conventional scales, neurologically distinct subtypes of apathy have been proposed. Here we compare the profile of apathy subtypes apathy in patients, and their carers, with Amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and Alzheimer's disease (AD).
Methods: 75 ALS, 47 AD, 27 PD patients and their carers completed the Dimensional Apathy Scale (DAS), composed of Executive, Emotional and Initiation subscales, a standard apathy scale- Apathy Evaluation scale (AES) and Geriatric Depression Scale (GDS15). There was no significant difference between patient group's years of education.
Results: Using the AES, generally ALS patients were less apathetic than AD patients and equally as apathetic as PD patients. A significant group (self rated) x subscale interaction effect was found, $F(4, 292)=3.590$, $p<.01$, showing that ratings on each DAS subscale differed in all three patient groups. Post hoc analysis showed that ALS patients had significantly lower scores on Executive apathy than both patients with PD, $t(100)=2.047$, $p<.05$, and AD, $t(120)=6.593$, $p<.001$. Additionally, ALS patients scored lower than AD patients on Emotional, $t(120) =6.630$, $p<.001$, and Initiation, $t(120)=4.753$, $p<.001$, apathy. Similarly, a significant group (carer rated) x subscale interaction effect was found, $F(4, 292)=6.714$, $p<.001$, with post hoc tests showing patients with ALS scored lower than AD on Executive, $t(100)=8.654$, $p<.001$, Emotional, $t(100)=5.656$, $p<.001$, and Initiation, $t(100)=5.537$, $p<.001$, apathy.
Conclusion: ALS patients were shown to have a different apathy profile from patients with AD and PD. ALS patients scored lower over all aspects of apathy compared to AD patients. Specifically they were found to have intact Executive motivation compared to both PD and AD patients. Future studies should comparatively explore multidimensional apathy in other neurodegenerative diseases populations.

OS2.3: COGNITIVE IMPAIRMENT IN ALS ASSESSED WITH 18F-FDG-PET *Umberto Manera, Antonio Canosa, Andrea Calvo, Barbara Iazzolino, Anna Montuschi, Cristina Moglia, Angelina Cistaro, Mauro Pagani, Adriano Chio'*

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Background: ~15% of ALS patients show a full-blown frontotemporal dementia (FTD), while ~35% have more subtle alterations. Our aim was to characterize the different patterns of cognitive impairment by 18F-FDG-PET in a large sample of ALS patients.
Methods: We performed neuropsychological assessment and 18F-FDG PET in 170 subjects with definite, probable or probable laboratory-supported ALS patients consecutively recruited at the ALS Centre of Turin (Italy). The cognitive classification was the following: patients with normal cognition (ALS-Cn), ALS-FTD, ALS with cognitive impairment (ALS-Ci, patients with deficit of two executive or two non-executive tests), ALS with behavioral impairment (ALS-Bi), ALS with non-classifiable deficits (ALS-NC). Group comparisons were performed by SPM8 among ALS-Cn (n=94), ALS-FTD (n=20), ALS-Ci (n=37), and ALS-Bi (n=9).
Results: Compared to ALS-Cn, ALS-FTD showed a large cluster of relative hypometabolism including bilateral premotor, frontal and anterior prefrontal cortex with left predominance as well as left lateral prefrontal and orbitofrontal cortex, while ALS-Ci had a hypometabolic cluster in right anterior cingulate, frontal and prefrontal cortex as well as in left prefrontal cortex. No difference was detected between ALS-Bi and any other group. The finding of hypometabolism in frontal regions was related to hypermetabolism in cerebellum, midbrain and corticospinal tracts in proportion to the degree of severity of the former.
Discussion: We confirmed at neurobiological level the neuropsychological evaluation, showing a significant reduction of frontal and prefrontal metabolism in ALS-FTD patients compared to ALS-Cn, and an intermediate metabolic pattern in ALS-Ci in frontal cortex, being hypometabolic compared to ALS-Cn and hypermetabolic compared to ALS-FTD. The finding of hypermetabolic clusters, mainly in white matter, in association with frontal hypometabolism suggests that astrocytosis might be involved in ALS-related neurodegeneration. The present study is the first one arising the possibility to discriminate ALS patients with different levels of cognitive deficits by 18F-FDG PET.

OS2.4: COGNITIVE DEFICITS IN PURE ALS: A DATA DRIVEN APPROACH *Mr. Tom Burke 1, 2; Dr. Marwa Elamin 2; Prof. Orla Hardiman 2, 3; & Dr. Niall Pender 1, 2, 4*

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Up to 50% of people with ALS are known to experience cognitive decline, with the co-occurrence of frontotemporal dementia in approximately 15% of these patients. Frontal-executive deficits represent the largest manifestation of cognitive impairment associated with ALS in patients with, and without the C9orf72 expansion. Our objective was to investigate the neurocognitive profile of Pure ALS patients, who represent patients with no cognitive abnormality detected on testing, and compare their results to controls. Patients completed a full neuropsychological assessment as part of a longitudinal investigation of cognitive phenotypes in ALS. Patients who were negative for known genetic mutations i.e., C9orf72, and who presented as Pure ALS on testing, were included (n=106), alongside age, gender, and education-matched controls (n=117). Neuropsychological data were collected through clinic based recruitment and home-based assessments. Preliminary cross-sectional cluster based analyses accurately categorized healthy controls, impaired participants and Pure ALS patients into three distinct groups, with 100% accuracy, based on cognitive outcome data. Pure ALS patients, though categorically unimpaired, performed significantly worse than controls on multiple measures of executive function, and also visuo-spatial encoding (p= .031). Executive processes included particularly sensitive measures such as fluency (p= .001), set shifting (p= .006), and working memory (p= .008). Our cluster analyses confidently show how Pure ALS patients scoring profiles are distinctively different to controls and impaired patients. Further findings suggest that ALS patients who are categorically identified as having no cognitive abnormalities may experience executive deficits. These data suggest the presence of a specific mild cognitive impairment in ALS patients, specific to executive function. These impairments may be considered as prodromal to cognitive impairment in ALS, with longitudinal implications to be discussed. This cognitive data-driven approach questions the weight of the term Pure ALS, and enforces the presence of subtle cognitive deficits secondary to ALS.

OS3.1: GENETIC CORRECTION OF C9ORF72 REPEAT EXPANSION MUTATION IN ALS/FTD PATIENT IPSCS *Nidaa Ababneh1,2, Rowan Flynn2, Ruxandra Mutihac1, Kenny Moore2, Martin Turner1, Salley Cowley2, Kevin Talbot1*

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder, characterized by the degeneration of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord, leading to paralysis and death within 2-5 years of onset. A hexanucleotide (G4C2) repeat expansion in chromosome 9 open reading frame 72

(C9ORF72) gene has been identified as a major cause of familial ALS and frontotemporal dementia (FTD). In this project, induced pluripotent stem cell (iPSC) lines were generated from four ALS patients carrying the repeat expansion mutation in C9ORF72 gene. Three iPSC lines were generated for each patient and each shown not to carry any major genomic instability. One ALS/FTD patient iPSC line (OXC9-02-02) was differentiated successfully into motor neurons and also used for gene editing to target the expanded G4C2 repeats using CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9-mediated homologous recombination (HR), in the presence of plasmid DNA donor template containing a puromycin cassette for positive selection of the corrected clones. Puromycin resistant clones were assessed by repeat-primed PCR (RP-PCR), and then evaluated for targeted integration by direct sequencing. Twenty four out of one hundred clones showed no repeat expansion by RP-PCR and positive results by direct sequencing and insertion of the wild type repeats. To the best of our knowledge, this is the first study of generating isogenic control for repeat expanded diseases using CRISPR technology, to understand the pathogenesis of the repeat expansion and to develop possible therapeutics. Taken together, these results demonstrate that the CRISPR/Cas9 system can be used for gene editing of repeat expansion mutation. This has the potential to produce a model system in which to discover potential therapeutics by illuminating basic disease mechanisms.

OS3.2: C9ORF72 REPEAT EXPANSIONS PRODUCE DISTINCTIVE METABOLIC PROFILES IN HUMAN CNS TISSUE *Gabriel N. Valbuena (1), J. Robin Highley (2), Janine Kirby (2), Pamela J. Shaw (2), and Hector C. Keun (1)*

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Hexanucleotide repeat expansions in the C9ORF72 gene have been identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS), and provides a previously unspecified link with cases of frontotemporal dementia. However, the function of the C9orf72 protein continues to be unclear, and while the repeat expansion has been shown to lead to possible haploinsufficiency, the formation of nuclear RNA foci sequestering RNA-binding proteins, and the formation of dipeptide repeat proteins, a definitive pathogenic mechanism for the disease remains elusive. To examine potential impacts of the disease on metabolic processes in the CNS, we employ a metabolic profiling approach using gas chromatography-mass spectrometry on cerebellum, frontal cortex, motor cortex, and thoracic spinal cord tissue from C9ORF72 ALS patients and compared profiles to tissue from sporadic ALS cases (sALS) and control individuals. We observe a characteristic metabolic profile for C9ORF72 ALS patients in the cerebellum after principal components analysis. Significant increases in amino acids including glutamate, threonine, and tyrosine, carboxylic acids including iminodiacetic acid and lauric acid, as well as metabolites linked to energy metabolism including lactate and alanine were observed in C9ORF72 ALS compared to control and sALS. Weaker separation of C9ORF72 ALS cases in the first principal component was seen in frontal cortex tissue, while a small difference in the second principal component was observed for sALS cases compared to both control and C9ORF72 ALS. Our results suggest that the repeat expansion may lead to metabolic changes that contribute to the

distinct profile observed in the cerebellum. Improved understanding of the metabolic alterations leading to the characteristic metabolomic phenotype observed may provide new insights on the processes involved in C9ORF72 ALS pathogenesis.

OS3.3: C9ORF72 EPIGENETIC MODIFICATIONS IN ITALIAN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS *Daniela Calini1,2, Cinzia Tiloca2, Federico Verde1,2, Elisa Onesto2, Davide Gentilini3, Nicola Ticozzi1,2, Antonia Ratti1,2, Vincenzo Silani1,2*

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The hexanucleotide repeat expansions (RE) in the non-coding region of C9orf72 are by far the most frequent cause of both familial and sporadic ALS and frontotemporal dementia. The basis for C9orf72 carriers clinical heterogeneity remains unknown. C9orf72 promoter methylation and histone trimethylation have been associated with transcriptional silencing and with decreased formation of RNA foci and dipeptide repeat protein aggregates. These findings suggest that epigenetic modification of C9orf72 could influence disease presentation. To consolidate this hypothesis we studied C9orf72 promoter methylation in our cohort of 44 ALS C9orf72-positive patients using bisulfite conversion and DNA sequencing. For the first time, we coupled Sanger sequencing to next generation sequencing (NGS) to estimate with extreme accuracy the percentage of methylation at each CpG site. As a result, C9orf72 promoter methylation was identified in 57% of C9orf72 carriers, but only in 20% of cases it was a widespread event involving nearly all 26 CpG sites analysed. This new approach revealed also that, relative to NGS, Sanger sequencing alone may lead to over-estimate samples methylation state. By performing Q-PCR on blood RNA, we found a positive correlation between C9orf72 promoter methylation and lower levels of total C9orf72 mRNA compared to both unmethylated C9orf72 carriers and C9orf72-negative patients, with a different expression profile of the three C9orf72 isoforms (V1, V2 and V3). While methylation levels didn't correlate with age at onset, they were mildly correlated with disease duration and inversely associated with RE length, determined by Southern blot. Hence our data support the current hypothesis that epigenetic silencing of mutant C9orf72 is associated to diminished levels of expanded mRNAs prone to generate pathologic effects and a less aggressive disease phenotype. However, since hydroxymethylation has an opposite effect on gene expression compared to methylation, we also plan to evaluate the contribution of this modification to C9orf72 expression.

OS3.4: DIAPHRAGMATIC PACING IN MOTOR NEURONE DISEASE: A RANDOMISED CONTROLLED TRIAL (DIPALS) *Dr Christopher J McDermott (on behalf of the DiPALS study Group), Reader in Neurology, Sheffield Institute for Translational Neuroscience (SITraN) University of Sheffield, Sheffield, UK*

Introduction: Non invasive ventilation (NIV) is the standard care for the treatment of respiratory failure in motor neurone disease (MND), providing both survival and quality of life gains for individuals. However NIV is not tolerated in all and becomes ineffective as the disease progresses. Diaphragm pacing (DP) is being increasingly used world wide, largely as an adjunctive treatment for respiratory failure in MND. Multiple case series have demonstrated operative safety and a cohort study led to FDA approval for DP as a treatment for respiratory failure in MND under the Humanitarian Device Exemption rules.
Objective: The primary objective was to evaluate the effect of Diaphragm Pacing (DP) on survival over the study duration. The secondary objectives were to evaluate safety and tolerability of DP, and the impact on quality of life of the individual with MND and their main carer.
Methods: A multi-centre prospective randomised controlled interventional trial. Patients were randomly allocated to receive either standard care (NIV) or standard care with additional DP.
Results: The study intended to recruit a total of 108 patients (54 per group) which would have given a power of 85% using a two-sided type I error of 5% to detect a 25% difference in survival between groups. In December 2013 the data monitoring ethics committee (DMEC) independently reviewed the survival data and advised the study team to put a hold on further implantations/ recruitment but to continue to actively treat patients in both groups. In August 2014 the DMEC advised all pacing to stop but for follow up to continue until the planned study end in December 2014. The data for the 77 patients recruited to DiPALS will be presented.

OS4.1: SIMULATING DISEASE SPREAD IN AMYOTROPHIC LATERAL SCLEROSIS USING A CONNECTOME-BASED MODEL *Ruben Schmidt, MSc1, Marcel A. de Reus, MSc2, Lianne H. Scholtens, MSc2, Leonard H. van den Berg, MD, PhD1, Martijn P. van den Heuvel, PhD2*

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of motor function. Aggregates of phosphorylated 43kDa TAR DNA-binding protein (pTDP-43) have been identified as a key component of neuronal inclusions in sporadic ALS patients, potentially an important trace of the pathogenic mechanism underlying disease progression. Here, we introduce a computational model to test the hypothesis of pTDP-43 aggregates to spread between brain regions across the white matter pathways of the connectome.
Our model of axonal disease spread, based on disease particles 'walking' along the connections of a network, simulates the self-perpetuating 'prion-like' property of misfolded TDP-43 proteins by new 'walkers' emanating from visited regions. Using the macaque

connectome, derived from gold standard tract-tracing experiments, we were able to include unique directionality information of tracts in in silico simulations of pTDP-43 spread. As an alternative to the hypothesis of axonal disease spread, levels of pTDP-43 aggregation were also evaluated using a model of spatial spread, not involving the connectome.
Simulations of axonal pTDP-43 spread show strong overlap with a recently proposed sequential neuropathology staging in ALS ($p = 0.02$), with the highest simulated levels of aggregation in first-stage motor-regions, followed by lower levels in subsequent stages two to four as defined by Brettschneider and colleagues. Simulated pTDP-43 aggregation levels using the spatial model, on the other hand, did not overlap with the sequential disease stages ($p > 0.9$), suggesting the axonal model better explains observed patterns of pTDP-43 pathology. Combining findings of neuropathology with a mammalian structural connectome map in a computational model, we show evidence of neuropathology spread in ALS to be guided by the underlying white matter connectome.

OS4.2: PHYSICAL DISABILITY IN ALS IS ASSOCIATED WITH FUNCTIONAL CONNECTIVITY CHANGES *Kristian Loewe* (1,2), Judith Machts* (1,3), Joern Kaufmann (1), Susanne Petri (4), Reinhard Dengler (4), Hans-Jochen Heinze (1,3,5), Christian Borgelt (6), Stefan Vielhaber (1,3), Mircea Ariel Schoenfeld (1,5,7)*
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Background: Previous studies on resting-state functional connectivity in ALS have identified patterns of altered connectivity within primary motor and extra-motor regions. However, the relationship between these changes and the progression of disability is unclear. Whole-brain voxel-level graphs were used to map functional connectivity changes associated with the physical disability measured by ALSFRS-R.
Methods: 64 patients with ALS underwent resting-state fMRI at 3T (TR = 2200ms, isotropic 3.5mm voxels). Subject-specific connectivity graphs were constructed by defining gray matter voxels as nodes and establishing weighted edges by estimating internodal functional connectivity between the nodes' associated time-series. Edge-level correlations with the patients' ALSFRS-R score were computed across graphs to assess connectivity changes associated with physical severity.
Results: Physical disability correlated with reduced connectivity within the bilateral sensorimotor cortices. In addition, the connectivity of these regions with frontal, parietal, inferior temporal, and occipital regions - mostly, but not exclusively, of the same hemisphere - showed marked reduction as a function of disease progression. The same pattern was found between the sensorimotor cortices and both hippocampi, which themselves exhibited a decreased connectivity with the amygdala, the occipital and the inferior temporal cortex, thalamus and the basal ganglia.

Increased connectivity associated with increasing disability was observed between the frontal cortices and bilateral parietal cortex, the basal ganglia, and the inferior temporal cortex. The parietal cortices also exhibited increased connectivity correlated with disability with the occipito-temporal cortex, basal ganglia and amygdala. Increased connectivity between the right hippocampus and the right parietal cortex also correlated with physical disability.
Discussion: ALS progression is accompanied by concurrent reductions in motor-related connectivity. Changes in hippocampal connectivity were also correlated with the disability, suggesting a key functional role of the hippocampus in disease progression (Stoppel et al., 2014). With higher disability, patients exhibited increased connectivity across the frontal and parietal cortices, possibly reflecting a loss of cortical inhibition.

OS4.3: PATHOLOGY SPREADING IN ALS: A SINGLE-CENTER IN VIVO DTI-BASED STAGING ANALYSIS IN 289 PATIENTS *Jan Kassubek, Hans-Peter Müller, Kelly Del Tredici, Dorothée Lulé, Annemarie Hübers, Jürgen Keller, Martin Gorges, Heiko Braak, Albert C. Ludolph*

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Introduction: Neuropathological studies in ALS have shown that ALS may disseminate in a regional sequence in four disease-related patterns [1]. The application of in vivo diffusion tensor imaging (DTI) analysis to fiber structures that are prone to be involved at each neuropathological pattern of ALS [2] in a large scale study sample including follow-ups.
Methods: Two data samples, consisting of 414 DTI data acquired at 1.5T and at 3.0T from ALS-patients (N=289) and from controls (N=125) were analyzed by a tract of interest (TOI)-based fiber tracking approach to analyze tracts 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), together with reference pathways. Thirty-seven ALS-patients obtained a follow-up scan with a time-interval of 6 months in average.
Results: The statistical analyses of TOIs by tractwise fractional anisotropy statistics [3] showed differences between ALS-patients and controls for all investigated tracts. Data analysis at the individual level allowed for a categorization into ALS patterns in plausible agreement with post mortem neuroanatomical studies. Out of the 37 longitudinal data sets, 3 ALS-patients showed an increase in ALS-stage, and 34 ALS-patients remained stable.
Discussion: In summary, the TOI-based technique allowed for individual analysis of predefined tract structures in the large sample of ALS-patients. That way, in vivo imaging of the disease patterns in ALS has become feasible, cross-sectionally and longitudinally. This approach might enlarge the spectrum of potential non-invasive surrogate markers as a neuroimaging-based read-out for clinical trials in ALS.
References
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OS4.4: IMAGING SIGNATURES OF ALS *Schuster C, Elamin M, Hardiman O, Bede P*

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Introduction: Despite the large number of recent imaging studies in ALS, many studies focus on fractional anisotropy and mean diffusivity as sole measures of white matter integrity. No consensus exists as to which diffusivity indices are the most sensitive for ALS-related white matter degeneration.
Objectives: 1: To describe a core, three-dimensional ALS-specific white matter signature in a large cohort of ALS patients. 2: To identify which white matter measures are most sensitive to capture ALS-related changes. 3: To highlight patterns of grey matter involvement in ALS. 4: To demonstrate the discriminating distribution of individual data sets for the identified regions.
Methods: A large, single-platform, single-protocol, multimodal neuroimaging study has been undertaken with 70 ALS patients and 40 age-matched healthy controls. Focal grey matter density differences were identified by voxel based morphometry; white matter changes were explored by Tract-based Spatial Statistics. Multiple diffusivity measures were analysed and juxtaposed; fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD), radial diffusivity (RD).
Results: Similarly to previous studies, ALS patients exhibit diffusivity cortical density changes in the precentral gyrus, superior temporal, and bilateral frontal lobes. White matter analyses confirmed extensive corticospinal tract degeneration in the mesencephalic cruri, posterior limbs of the internal capsules, and in the coronae radiatae bilaterally. Interestingly, white matter degeneration has also been identified in the bilateral fornices, in the anterior body and the genu of the corpus callosum. RD was the most sensitive to highlight ALS-related white matter changes.
Conclusions: Radial diffusivity best discriminates between ALS patients and healthy controls suggesting a degree of myelin-related change in addition to the axonal degeneration demonstrated by widespread axial diffusivity changes. Fractional diffusivity alone is a suboptimal proxy of ALS-related white matter change. Fornix integrity is significantly affected in ALS which may contribute to the amnestic deficits observed clinically.

OS 5.1: PTDP43 IS A BETTER CORRELATE OF CLINICAL PHENOTYPE THAN RNA FOCI OR GA DIPEPTIDE IN C9ORF72 ALS/FTD *Jakub Scaber, Nuffield Department of Clinical Neurosciences, University of Oxford;Dirk Bäumer, Department of Neurology, Southampton General Hospital;Monika Hofer, Nuffield Department of Clinical Neurosciences, University of Oxford;Kevin Talbot, Nuffield Department of Clinical Neurosciences, University of Oxford; Olaf Ansorge, Nuffield Department of Clinical Neurosciences, University of Oxford;*

Background: The C9orf72 hexanucleotide expansion is the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). It is characterised by phospho-TDP-43 pathology but also features specific to the disease, which include RNA foci and aggregations of dipeptide protein. The relative contribution of these three pathologies to disease phenotype remains controversial.
Methods: We obtained brain tissue from ten patients with the C9orf72 expansion, five with sporadic ALS and five controls without CNS disease from a clinically well-characterised cohort. Of the C9orf72 patients, six were diagnosed with ALS, three with ALS-FTD and three

with pure FTD. We examined paraffin embedded sections from five brain regions: non-motor frontal cortex, hippocampus, cerebellum, medulla, spinal cord. We characterised the distribution of p62, phospho-TDP and GA dipeptide pathology in neuronal cells using immunohistochemistry and carried out fluorescence in situ hybridisation for sense RNA foci using a Cy3-labelled (CCCCGG)4 probe. RNAse and DNAse treatment confirmed the specificity of the probe. We carried out correlative analyses of clinical phenotype with pTDP, GA peptide and RNA sense foci.
Results: In our cohort, the brain areas affected by pTDP correlated best with the clinical diagnosis. GA dipeptide inclusions were not seen in the XIIth nerve nuclei or anterior horns of the spinal cord, even in pure C9orf72 ALS cases, and were ubiquitously expressed in the remaining areas studied. There was a statistically insignificant trend for increased GA dipeptide inclusions in the hippocampus of FTD patients. RNA foci were present in all brain areas studied and did not vary between disease phenotypes.

Conclusion: In our experimental setting, phospho-TDP43 pattern correlated better with clinical phenotype in C9orf72 carriers than GA peptide and sense RNA foci. This emphasises the importance of pTDP-43 as a common pathway driving the ALS and FTD phenotypes in sporadic and C9orf72 disease.

OS 5.2: PRIMARY FIBROBLASTS CULTURES REVEAL TDP-43 ABNORMALITIES IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS *Mario Sabatelli (1), Marcella Zollino (2), Amelia Conte (1), Alessandra Del Grande (1), Giuseppe Marangi (2), Matteo Lucchini (1), Massimiliano Mirabella (1), Angela Romano (1), Roberto Piacentini (3), Giulia Bisogni (1), Serena Lattante (2), Marco Luigetti (1), Alice Moncada (2)*

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TDP-43 is a major component of the pathological inclusions observed in the motor neurons of Amyotrophic Lateral Sclerosis (ALS) patients. We examined TDP-43 expression in primary fibroblasts cultures from 22 ALS patients, including cases with SOD1 (n.4), TARDBP (n.4), FUS (n.2), C9ORF72 (n.3) mutations and nine patients without genetic defect. By using a phosphorylation independent antibody, we detected quantitative changes of TDP-43 in the nuclear/cytoplasmic compartments of fibroblast from a group of fifteen ALS patients, with notable differences among cases. In one group, including patients with TARDBP mutations, TDP-43 was markedly increased in the cytoplasm and slightly reduced in the nuclei, resulting in a greater protein level in the cytoplasmic compartment than in the nuclear one. A different pattern was observed in C9ORF72 mutant cells in which a marked increase of TDP-43 was found in both nuclei and cytoplasm. A third pattern was that observed in ALS/FTD cases without genetic defects in which an abnormal level of TDP-43 the cytoplasm was not associated with significant nuclear changes. Finally, mutants SOD1 fibroblasts revealed a noticeable reduction of TDP-43 content in the nuclear fraction without cytoplasmic mislocalizzation. By using anti pTDP antibody, the totality of patients showed homogenously dispersed granular material in the cytoplasm and in occasional nuclei at confocal microscopy and this result was replicated in an independent cohort of 16 ALS patients. Our findings show that fibroblasts from ALS patients recapitulate some of hallmark TDP-43 abnormalities

observed in neuronal cells. Though TDP-43 appears to be differentially processed in fibroblasts versus neuronal cells from ALS patients, primary fibroblast cultures may represent a helpful tool to investigate TDP-43-mediated disease mechanisms. The evidence that SOD1 mutation clearly alter TDP-43 metabolism in fibroblasts is a novel finding and strongly support the hypothesis of an interaction between TDP-43 and mutant SOD1.

OS 5.3: DECIPHERING THE PATHOGENIC MECHANISMS OF TDP-43 AND FUS PROTEINOPATHIES IN NOVEL EX VIVO MODELS *Eva-Maria Hock1, Zuzanna Maniecka1, Marc Ruepp2, Manuela Neumann3, Simone Hornemann4 and Magdalini Polymenidou1*

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As proposed for many other neurodegenerative diseases, ALS and FTLD have been suggested to progress through a prion-like mechanism, based on self-templated seeded aggregation of pathogenic proteins, which trigger neurotoxicity and eventually neuronal cell death. TDP-43 and FUS, two major components of pathological inclusions in ALS and FTLD, share striking functional and structural similarities. Most importantly, both proteins contain low-complexity prion-like domains, which are highly prone to aggregation. Moreover, these proteins comprise multiple nucleic acid binding motifs and are implicated in several RNA metabolism pathways. While in healthy cells, TDP-43 and FUS localize predominantly to the nucleus, in affected neurons and glial cells, they mislocalize to the cytoplasm, where they form pathogenic inclusions. This protein redistribution leads to TDP-43 and FUS depletion from the nucleus, which suggests two potential disease mechanisms; namely, loss of normal nuclear function and gain of toxicity due to deposition of the misfolded protein assemblies in the cytoplasm. To recapitulate the pathology and study up- and downstream events of protein mislocalization, aggregate formation, toxicity and cell-to-cell spreading, we use exogenous FUS and TDP-43 seeds to trigger redistribution and aggregation of endogenous protein in mouse organotypic slice cultures. In preliminary experiments, we observed that the TDP-43 seeds were internalized by glial cells and were able to triggered phosphorylation of endogenous TDP-43, which resembles phosphorylation pattern in ALS cases. Moreover, as stress-induced protein translocation to the cytoplasm has been hypothesized to lead to aggregation in disease, we facilitate and/or initiate TDP-43 and FUS aggregation by chronic exposure of the slices to sublethal doses of various cell stressors, with or without application of reconstituted seeds. First studies with induced hyperosmolar stress, showed successful redistribution of FUS to the cytoplasm and its incorporation into stress granules.

OS 5.4: CELL-TO-CELL TRANSMISSION OF TDP-43 ACROSS AXON TERMINALS

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Causative mutations in TDP-43-encoding gene TARDBP were identified in fALS and sALS patients. Furthermore, TDP-43 is an aggregation-prone prion-like domain-containing protein and component of pathological intracellular aggregates. TDP-43 oligomers have been postulated to be released and subsequently nucleate TDP-43 oligomerization in recipient cells, which might be the molecular correlate of the systematic symptom spreading observed during ALS progression. We developed a novel protein complementation assay allowing quantification of TDP-43 oligomers in living cells. This assay is based on the principle of using non-bioluminescent Gaussia princeps luciferase fragments fused to TDP-43. We demonstrate the presence of TDP-43 oligomers in microvesicles/exosomes and show that microvesicular TDP-43 is preferentially taken up by recipient cells, where it exerts higher toxicity than free TDP-43. Moreover, using nanotechnology-derived microfluidic devices that fluidically isolate neuronal cell bodies from axon terminals in culture, we demonstrate both anterograde and retrograde trans-synaptic transmission of TDP-43. Finally, we demonstrate TDP-43 oligomer seeding by TDP-43 derived from both, cultured cells and ALS patient-derived CNS tissue extracts. Thus, using an innovative detection technique, we provide evidence for preferentially microvesicular and bidirectional synaptic intercellular transmission and prion-like seeding ability of TDP-43 oligomers.

OS6. 1: DIFFERENT REQUIREMENTS FOR THE VCP CO-FACTORS NPL4 AND UFD1 IN NEURONAL FUNCTION

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Neuronal aggregates containing ubiquitinated and disease-causing mutant proteins, often the RNA-binding protein TDP-43, are a common feature of amyotrophic lateral sclerosis (ALS). This suggests that the ubiquitin-proteasome system (UPS), the primary system responsible for the maintenance of protein turnover, is a critical factor in the pathogenesis of ALS. Supporting this, mutations in several genes encoding proteins which function in the UPS have been shown to cause ALS, most notably valosin-containing protein (VCP). Recent reports have proposed increasing the function of VCP as a therapeutic strategy to treat ALS. However, VCP functions in various cellular pathways, with the action of VCP determined by its association with different protein co-factors, e.g. VCP-Npl4-Ufd1 complexes function in UPS while VCP-p37 functions in golgi/ER biogenesis. Therefore, therapeutic strategies aimed at stimulating the UPS should likely be targeted to the VCP-Npl4-Ufd1 complex, which is poorly understood in neurons.

We have characterised the roles of Npl4 and Ufd1 in neurons in vivo by targeted gene knockdown in the fruit fly Drosophila melanogaster. Neuronal-specific knockdown of Npl4, but not Ufd1, results in widespread neurodevelopmental disruption. Specifically, analysis of motor neurons in Npl4 RNAi larvae reveals disrupted microtubule organisation within axon fibers and at the neuromuscular junction (NMJ). Furthermore, neuronal loss of Npl4, and to a lesser extent Ufd1, causes progressive degeneration with adult flies displaying reduced survival (Npl4 RNAi) and locomotion (Npl4 and Ufd1 RNAi) compared to controls. We next investigated how loss of these genes modified TBPH (Drosophila ortholog of TDP-43) expression in neurons. We identified increased cytoplasmic mislocalisation of TBPH in Npl4 RNAi neurons, consistent with the hypothesis that defects in UPS may underpin the pathogenic processes that lead to neurodegeneration in ALS. We have therefore generated a novel in vivo model in which to investigate the mechanism(s) by which UPS dysfunction contributes to ALS-associated neurodegeneration.

OS6. 2: SOD1G93A AFFECTS THE INTEGRITY AND ALTERS CLAUDIN 5 EXPRESSION IN A BLOOD-SPINAL CORD BARRIER MODEL *Sabrina Meister 1, Steffen E. Storck 1, Erik Hameister 1, Christian Behl 1, Sascha Weggen 2, Albrecht M. Clement 1, Claus U. Pietrzik 1*

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive paralysis due to the loss of primary and secondary motor neurons. Mutations in the Cu/Zn-superoxide dismutase (SOD1) gene are associated with familial ALS and to date numerous hypotheses for ALS pathology exist including impairment of the blood-spinal cord barrier. In transgenic mice carrying mutated SOD1 genes a disrupted blood-spinal cord barrier as well as decreased levels of tight junction (TJ) proteins ZO-1, occludin and claudin-5 were detected. Here, we examined TJ protein levels and barrier function of primary blood-spinal cord barrier endothelial cells of presymptomatic hSOD1G93A mice and bEnd.3 cells stably expressing hSOD1G93A. In both cellular systems we observed reduced claudin-5 levels and a decreased transendothelial resistance (TER) as well as an increased apparent permeability. Analysis of the β-catenin/AKT/forkhead box protein O1 (FoxO1) pathway and the FoxO1-regulated activity of the claudin-5 promoter revealed a repression of the claudin-5 gene expression in hSOD1G93A cells which was depended on the phosphorylation status of FoxO1. These results strongly indicate that mutated SOD1 affects the expression and localization of TJ proteins leading to impaired integrity and breakdown of the blood-spinal cord barrier.

OS6. 3: CHARACTERISATION AND MOTONEURONAL DIFFERENTIATION OF HUMAN FUS-ALS INDUCED PLURIPOTENT STEM CELLS *Julia Higelin, Ulm University*

Mutations in FUS (Fused in sarcoma) which is a multifunctional RNA/DNA-binding protein have been identified as a genetic cause for motoneuron (MN) degeneration. FUS is involved in processes of gene expression such as splicing, translation and transport of mRNA. In addition to its predominant localisation in nucleus, FUS presents a dendritic localisation and is detectable in synaptic spines in neurons. Recently, it has been demonstrated that FUS is implicated in DNA damage response (DDR) by being one of the factors mediating DNA repair

in neurons and by showing a direct interaction with Histone deacetylase 1 (HDAC1). Furthermore, fALS related mutations in FUS interfere with this interaction and therefore might lead to increased DNA damage in neurons.
Based on the described role of FUS in DDR we analyzed the reaction of FUS-specific human induced pluripotent stem cells (hiPSCs) to DNA-damage. For this, DNA breaks were induced by irradiation in hiPSC cells from a healthy control and a patient carrying a FUS mutation. Subsequently, hiPSC were morphologically analysed. Our first results indicate that our FUS-hiPSC cell line was more sensitive to DNA damage as compared to the control, since the number of apoptotic colonies versus the number of healthy colonies increased in the FUS mutated cells after 24 h.
Based on the ability of hiPSC derived MN to show a specific patient phenotype, we investigated FUS localisation in hiPSC derived MN. Our first data indicates that in control and patient-specific MN, FUS was predominantly localised in the nucleus but additionally FUS was also co-localising with synaptic markers. Moreover, only in MN from this ALS-patient, FUS also presented a cytoplasmic mislocalisation together with a strong puntae mislocalisation along the neurites.
Characterisation of other FUS mutations will reveal the specificity of FUS to DNA damage/repair or during other types of stress, and precise localisation of FUS will reveal the exact FUS mislocalisation seen firstly in this FUS hiPSC derived MN.

OS6. 4: MACROPHAGE MIGRATION INHIBITORY FACTOR AS A CHAPERONE INHIBITING ACCUMULATION OF MISFOLDED SOD1 *Adrian Israelson1, Salah Abu-Hamad1, Guy Zoltsman1, Tom Shani1, Dara Ditsworth2, Sandrine Da Cruz2, and Don W. Cleveland2*

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Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by loss of motor neurons and accompanied by accumulation of misfolded SOD1 onto the cytoplasmic faces of intracellular organelles, including mitochondria and endoplasmic reticulum (ER). Using inhibition of misfolded SOD1 deposition onto mitochondria as an assay, a chaperone activity abundant in non-neuronal tissues is now purified and identified to be the multifunctional macrophage migration inhibitory factor (MIF), whose activities include an ATP-independent protein folding chaperone. Purified MIF is shown to directly inhibit mutant SOD1 misfolding. Elevating MIF in neuronal cells suppresses accumulation of misfolded SOD1 and its association with mitochondria and ER and extends survival of mutant SOD1-expressing motor neurons. Accumulated MIF protein is identified to be low in motor neurons, implicating correspondingly low chaperone activity as a component of vulnerability to mutant SOD1 misfolding and supporting therapies to enhance intracellular MIF chaperone activity.

OS 7.1: PHARMACOLOGICAL CORRECTION OF DYSFUNCTIONAL MNS DIFFERENTIATED FROM ALS PATIENT-DERIVED IPSC *Maximilian Naujock 1,4, Nancy Stanslowsky 1, Sebastian Bufler 1, Andreas Hermann 2, Marcel Naumann 2, Peter Reinhardt 3, Jared Sterneckert 3, Kwang-Soo Kim 4, Florian Wegner 1 and Susanne Petri 1*

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For the study of functional deficiencies in neurodegenerative diseases patient-derived iPSC can be used as in-vitro disease models. As proof of principle, it has been demonstrated that motor neurons (MNs) differentiated from ALS-iPSC indeed recapitulate certain disease-specific abnormalities.
Based on a novel approach starting from expandable neural precursor cells generated from iPSC, we successfully differentiated five ALS-iPSC lines (n=3 patients) and five controls lines (n=3 healthy subjects) into Tuj1/Map2/SMI32/Islet1-positive MNs without observing major differences in differentiation efficiency. Patient- and control-derived MNs showed similar basic neural properties such as steady membrane potential and Na+K+ currents under patch clamp analysis. No significant differences between FUS and control lines were observed regarding the percentage of cells that were spontaneously active and they similarly fired single action potentials upon stimulation. However, in healthy controls we observed significantly higher frequencies of spontaneously occurring action potentials and postsynaptic miniature events, both of which are physiological parameters for spontaneous activity. Furthermore, upon stepwise depolarization a significantly higher percentage of control-cells responded with trains of action potentials.
In line with a very recently published study in mutant TDP-43 and C9orf72 MNs, our results indicate that ALS patient-derived MNs with mutations in the FUS gene present a hypoexcitability phenotype. At the same time these hypoexcitable FUS mutant cells present themselves with a significantly lower Na+K+ ratio. We therefore tested the benefits of the FDA approved drug 4-Aminopyridine (4AP) in its ability to antagonize the potassium currents which are held to be at least partly responsible for the observed hypoexcitability. 4AP incubation of mutant FUS MNs not only corrected the Na+K+ ratio back to control levels but also increased their excitability and spontaneous activity. Whether this treatment is neuroprotective and how exactly the hypoexcitable phenotype is related to the observed degeneration of MNs in ALS awaits further investigation.

OS 7.2: NEUROMUSCULAR JUNCTION FORMATION USING HIPSC CO-CULTURE SYSTEM: RELEVANCE TO AMYOTROPHIC LATERAL SCLEROSIS *Maria Demestre, Ulm University*

Striated skeletal muscle cells represent a valuable source for in vitro studies of the motoric system as well as for clinical implications. In amyotrophic lateral sclerosis (ALS) for example, upper and lower motorneurones (MN) degenerate leading to muscular atrophy and respiratory failure. However, in ALS there is not only MN pathology but also skeletal muscle is affected more in particular at the neuromuscular Junction (NMJ). Myoblasts can readily be grown from muscle tissue. If muscle tissue is unavailable, myogenic cells can be generated from human induced pluripotent stem cells (hiPSCs). We had previously established hiPSC

derived MN and currently we have generated and characterised hiPSC derived myogenic cells by PAX7 induction. Following the expansion of PAX7 expressing cells in myogenic culture conditions, serum deprived myoblast-like cells fused and formed multinucleated striated myotubes that expressed a set of key markers for muscle differentiation. In addition, these myotubes contracted upon electrical stimulation, responded to acetylcholine (Ach) and were able to generate action potentials. Finally, we co-cultured MN and myotubes generated from identical hiPSCs from two healthy controls. We could observe the early aggregation of Acetylcholine receptors in muscle cells of immature co-cultures. At later stages, we identified and characterised mature NMJs. On the other hand, in MN-myotube co-cultures generated from hiPSC from a patient carrying a Fused in Sarocoma (FUS) ALS related mutation NMJ did not fully mature, suggesting FUS mutation may impede proper NMJ formation. In summary, we describe here the successful generation of a functional cellular system consisting of two distinct communicating cells types in healthy and in ALS related mutations.

OS 7.3: PRESYMPTOMATIC ACTIVATION OF THE PDGF-CC PATHWAY ACCELERATES ONSET OF ALS NEURODEGENERATION *Sebastian A. Lewandowski1, Ingrid Nilsson1, Peter Lönnerberg2, Linda Fredriksson2, Lars Muhl1, Manuel Zeitelhofer1, Daniel A. Lawrence3, Eva Hedlund4, Ulf Eriksson1*
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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with unknown origins and limited options for therapy. Neurodegeneration in ALS occurs together with signs of disrupted blood-spinal cord barrier (BSCB) and regressed capillary network, but the molecular pathways contributing to the vascular defects remain unknown. Here we show that BSCB dysfunction in the SOD1G93A mouse model of familial ALS and in human sporadic patients can be caused by presymptomatic activation of the PDGF-CC pathway. Decrease of PDGFC expression restored vascular barrier properties in SOD1G93A mice and was correlated with delayed age of disease onset in transgenic mice and human sporadic ALS. However, restoration of BSCB integrity did not prevent the disease-induced capillary regression and lower vessel density was present in sporadic ALS patients and correlated with shorter disease duration after onset in SOD1G93A mice. We conclude that ALS onset and severity may be driven by two independent vascular events, which could contribute to the development of targeted novel therapies.

OS7.4: ROLE OF DYNEIN IN THE CLEARANCE OF MISFOLDED PROTEINS RESPONSIBLE FOR ALS *R. Cristofani1, V. Crippa1, M.E. Cicardi1, M. Meroni1, M. Galbiati1, P. Rusmini1, A. Poletti1*

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Amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA) share several similarities including the formation of aggregates of proteins with aberrant conformation (misfolded). Mutated SOD1, TDP-43, FUS-TLS, the C9ORF72 dipeptide, etc. in ALS and mutated androgen receptor (AR) in SBMA are misfolded proteins that aggregate. In affected motoneurons, the protein quality control (PQC) system (chaperones, autophagy and ubiquitin-proteasome system (UPS)) protects from misfolded proteins toxicity. The HSPB8-BAG3-HSC70-CHIP complex promotes aggregate degradation by autophagy.
BAG3 interacts with dynein transport of mutant proteins at microtubule organization center (MTOC) where they aggregate, and degraded by autophagy. However, here misfolded proteins may impair autophagy causing flux blockage.
In NSC34 cells we evaluated the role of dynein into aggregates formation process. Unexpectedly, treatment with a dynein inhibitor (EHNA) drastically reduced the retention of mutSOD1, mutTDP43 and mutAR aggregates in filter retardation assay (FRA), even when autophagy was inhibits with 3-MA. Conversely, UPS blockage with MG132 counteracted the reduction induced by altered dynein transports. In addition, the silencing of dynein heavy chain resulted in a drastic reduction of mutAR retained in FRA. Moreover, dynein silencing drastically altered localization of autophagic markers (LC3 and p62). RTq-PCR on RNAs from NSC34 cells treated with EHNA showed an increased BAG1:BAG3 ratio to re-route misfolded proteins to UPS. Moreover, EHNA increased the degradation of proteasome reporter GFPu, while BAG1 overexpression reduced the level of aggregates retained in FRA.
Collectively, these data suggest that when autophagy is overload by misfolded proteins, dynein inhibition restores the physiological and soluble protein pool via UPS.
GRANTS: Telethon, AriSLA, Cariplo, Regione Lombardia, UniMi, Ministero della Salute (Italy); AFM (France)

OS8.1: CHARACTERISING THE METABOLIC PROFILE OF ALS: RESULTS FROM THE EUROMOTOR STUDY COHORT *Alexandros P Siskos1, Orla Hardiman3, Adriano Chio4, Ettore Beghi5, Giancarlo Logroscino6, Jan Veldink2, Leonard van den Berg2$, Hector Keun1*

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On behalf of the EuroMotor consortium.

Amyotrophic Lateral Sclerosis (ALS) a devastating disease affecting some 50,000 individuals at any time in Europe. Currently, there is no cure for ALS and a lack of validated targets

reflects our inadequate understanding of disease mechanism and progression. The aim of the FP7 Euro-MOTOR study is to identify novel causes of ALS using a comprehensive systems biology approach. Within this study a large-scale, pan-European population-based metabolomic study has been conducted. The EuroMotor cohort comprises 1600 individuals (800 cases and 800 controls), from the Netherlands, Italy and Ireland. The patient cases are matched to controls for sex and age (approximately 5 years). We have carried out metabolomic analysis on serum from a discovery sub-cohort of 400 case-control pairs using the targeted AbsoluteIDQTM p180 platform (Biocrates Life Scieneces AG). The AbsoluteIDQTM p180 kit allows the targeted analysis of amino acids, biogenic amines, acylcarnitines, sphingolipids and glycerophospholipids. Our initial analyses identify major differences in the overall profile of carnitines, amino acids and lipids between patients and matched controls. In patient serum samples we observed a statistically significant decrease of the ratio (C2+C3 carnitine)/carnitine, and also increase of carnitine, indicating a perturbation of β-oxidation activity in patients. Total amino acids, branched chain amino acids, aromatic amino acids and also essential amino acids all appear to be significantly reduced in ALS cases compared to matched controls. The ratios of polyunsaturated fatty acids (PUFA) to monounsaturated fatty acids (MUFA) and MUFA to saturated fatty acids (SFA) in phosophatidylcholines (PCs) was reduced, whereas the PC-SFAs in patients appeared to be increased compared to controls. Thus, the saturation level of fatty acids appears to be higher in patients possibly due to differences in activity of fatty acid desaturases or nutritional contributions to lipid composition.

OS8.2: SERUM MICRORNAS IN SPORADIC AMYOTROPHIC LATERAL SCLEROSIS *Axel Freischmidt (a), Kathrin Müller (a), Lisa Zondler (a), Patrick Weydt (a), Benjamin Mayer (b), Christine A.F. von Arnim (a) , Johannes Dorst (a), Markus Otto (a), Karlheinz Holzmann (c), Albert C. Ludolph (a), Karin M. Danzer (a), Jochen H. Weishaupt (a)*

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Growing evidence implicates mRNA, but most recently also microRNA (miRNA) dysmetabolism in disease pathogenesis of amyotrophic lateral sclerosis (ALS). MiRNAs are post-transcriptional regulators of gene expression and specific miRNA “fingerprints” are thought to contribute to and/or reflect certain disease conditions. Recently, we identified surprisingly homogeneous signatures of circulating miRNAs in the serum of familial ALS patients, which were already present in pre-symptomatic carriers of ALS gene mutations and largely independent of the underlying disease gene. Here we characterize circulating miRNAs in the serum of sporadic ALS patients. In contrast to familial ALS, miRNA profiles of sporadic ALS are highly heterogeneous suggesting a number of different etiologies. Nevertheless, two miRNAs, miR-1234-3p and miR-1825, could be identified to be consistently downregulated in sporadic ALS. Both miRNAs may be active in the same functional network with largely overlapping target genes. Bioinformatic analysis revealed miRNA fingerprints resembling those of familial ALS patients and mutation carriers in 61% of sporadic ALS patients, while the remaining subgroup had clearly different miRNA signatures. These data thus suggest a higher than expected contribution of genetic factors also to

sporadic ALS. Moreover, our results indicate a more heterogeneous molecular etiology of sporadic ALS compared to (mono) genic cases, which should be considered for the development of disease modifying treatments.

OS8.3: BRAIN MORPHOLOGICAL CHANGES IN ASYMPTOMATIC 9ORF72 REPEAT EXPANSION CARRIERS *Renée Walhout MD(1), Ruben Schmidt MSc(1), Henk-Jan Westeneng MD(1), Esther Verstraete MD PhD(1), Meinie Seelen MD(1), Wouter van Rheenen MD(1), Marcel A. de Reus MSc(2), Michael A. van Es MD PhD(1), Jeroen Hendrikse MD PhD(3), Jan H. Veldink MD PhD(1), Martijn P. van den Heuvel PhD(2)†, Leonard H. van den Berg MD PhD(1)†*

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Objective: The C9orf72 repeat expansion is an important cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Asymptomatic carriers of C9orf72 provide a unique opportunity to study possible effects of the repeat expansion before disease onset. In this study, brain morphology of asymptomatic carriers was investigated by neuroimaging.
Methods: Aiming to diminish the effects of genetic variation between subjects, apart from the C9orf72 repeat expansion, 16 carriers of the repeat expansion were compared to 23 non-carriers from the same large family with a history of ALS. Cortical thickness, subcortical volumes and white matter connectivity, as assessed from high resolution T1-weighted and diffusion-weighted magnetic resonance images, were evaluated. For comparison, 14 C9orf72 ALS patients and 28 age- and gender-matched healthy unrelated controls were included.
Results: We found temporal, parietal and occipital regions to be thinner ($p < 0.05$) and the left caudate to be smaller ($p < 0.05$) in asymptomatic carriers compared to non-carriers. Cortical thinning of the primary motor cortex and decreased connectivity of white matter pathways (global, corticospinal tract and corpus callosum) were observed in C9orf72 ALS patients, but not in asymptomatic carriers.
Conclusions: Asymptomatic C9orf72 carriers show cortical and subcortical differences compared to non-carriers from the same family, suggesting these are effects of the C9orf72 repeat expansion on the brain. Notably, changes in the primary motor regions and motor-related tracts were found exclusively in patients with ALS, indicating that such motor changes may be a disease phenomenon.

OS8.4: ABNORMAL BETA-BAND DESYNCHRONISATION AS A PRE-SYMPTOMATIC BIOMARKER OF MOTOR NETWORK DYSFUNCTION

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Background: Abnormalities in cortical network activity have been revealed by functional MRI to be potentially early markers of neurodegeneration. The distortion-free signals of continual neuronal oscillations measured by magnetoencephalography (MEG) offer exquisite temporal sensitivity.
Objectives: We used MEG to study the cortical networks sub-serving motor preparation, execution and inhibition in affected ALS and PLS patients, and pre-symptomatic individuals at high genetic risk of ALS.
Methods: Nineteen affected patients (11 ALS, 8 PLS), 12 pre-symptomatic mutation carriers (10 SOD1, 2 C9orf72) and 14 controls participated. Action preparation and completion was studied during MEG acquisition using a cued go/no-go task requiring responses with either index finger. A central visual cue incorporated both spatial (left or right hand for response) and temporal (variable interval from cue to target) information regarding the subsequent target appearance, with 100% and 80% validity respectively. Task performance was checked off-line from surface EMG recordings made over the forearm extensors. Data were analysed using a locally developed toolset based upon similar principles to functional MRI analysis.

Results: Both ALS and PLS patient groups had slower mean reaction times compared to controls; the pre-symptomatic mutation carriers' mean reaction times were faster, but made more no-go errors (27% versus 16%). All patients and pre-symptomatic individuals displayed excessive beta-band desynchronisation, particularly ipsilateral to the effector limb, and with attenuated beta rebound. PLS patients showed the most marked abnormality. Source reconstruction to a standard MRI template mapped these abnormalities to the precentral gyrus. Qualitatively similar, but smaller effects were seen in the pre-symptomatic group.
Conclusions: The ipsilateral hemispheric beta-band changes may be more specifically linked to degeneration of corpus callosum fibres, most marked in PLS. These novel findings should encourage further development of MEG as a biomarker tool, with potential to elucidate both pathological and compensatory cortical motor network processes, including pre-symptomatic changes.

Poster Session I
Thursday 21st May, 17.30-19.00 (Chairs:)

CLINICAL
P.01: PERCEIVED QUALITY OF LIFE IN AN ALS MULTIDISCIPLINARY CARE UNIT. MULTIDOMAIN ANALYSIS AND FURTHER EVIDENCE OF THE "DISABILITY PARADOX"

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Due to the invalidating nature of ALS, patients frequently loss function on communication, mobility and feeding skills. This can result in significant emotional distress. The relationship between these factors is poorly understood.
Objectives: To understand the quality of life in our population and its repercussions on emotional wellbeing.
Methods: ALSAQ-40 is a well-validated instrument that evaluates both physical and emotional areas considered important for ALS patients.
Results: We evaluated a total of 73 patients, 36 female 37 male. 17,8% had bulbar onset and 82,2 had a spinal onset. Mean age at diagnosis was 61 years old (range 34–85). Mechanical ventilation was present in 50,7% of cases and 31,5% had gastrostomy. We classified patients according with time from onset as initial (>12 months), intermediate (12 – 36 months) or late disease (>36 months). Emotional distress had a significant correlation with mobility, independence, communication and feeding indexes. The strongest correlation was with mobility and communication (Pearson 0,49 and 0,35 respectively, p>0,01).
Other important correlations were that of mobility and independence (Pearson 0,71, p>0,001) and communication and feeding (Pearson 0,67, p>0,001).
As expected, both mobility and independence worsened amongst early, intermediate and late disease, although without reaching statistical significance (p= 0,126). However, emotional index remained stable, a finding consistent with what has been called the "disability paradox".
Use of mechanical ventilation was related with slightly elevated scores of independence (71 vs. 61, p=0,12) and communication indexes (52 vs 42 p=0,17) without reaching significance. Neither use of ventilation nor gastrostomy had any impact on emotional indexes.
Conclusion: ALS affects quality of life because of its multidomain nature. While emotional repercussions are initially related with physical disability, it remains stable through disease, suggesting either coping or other psychosocial factors are involved.

P.02: EVALUATION OF THE CAREGIVER BURDEN USING THE ZARIT INTERVIEW SCORE IN AN ALS MULTIDISCIPLINARY CARE *Tejado A, Paipa A, Verges E, Martinez Y, Begoña A, Turon J, Povedano M.*

ALS Multidisciplinary Care Unit, Hospital Universitari de Bellvitge, L'Hospitalet de llobregat, Spain.

Introduction: Care of ALS patients can be challenging because of its progressive and invalidating nature. This can result in an emotional distress for family members and other caregivers at home.
Objectives: We sought to evaluate the incidence and severity of emotional distress using the Zarit Burden Interview scale. We also wanted to describe posible clinical modifiers or precipitating factors.
Methods: During a three month period we completed the Zarit Interview test on family members or caregivers for every patient who atended our ALS clinic. Clinical characteristics had been previously obtained in a centralized database as per protocol.
Results: We analized a total of 65 caregivers (50,8% male 49,2% female). Mean age was 61,5 years (range 38 – 62) onset was spinal in 80% and bulbar in 20%. 39 caregivers (60%) did not report burden of care (Zarit Score <47). 12 (18,5%) had a mild to moderate burden (Zarit 47 – 55) and 14 (21,5%) an intense burden.
The proportion of caregivers with no signs of overload was similar amongst age groups, type of onset and use of mechanical ventilation. Amongst those caregivers who reported burden symptoms (n=26), older age related with higher severity of distress (66,7% on intense burden group amongst 65 years old or older vs 42% on patients under 65, p = 0,26) as well as spinal onset (60% vs 33%, p= 0,36) and need for mechanical ventilation (80% vs 37,5% of patients with intense burden). While similar intense burden was reported amongst genders, caregivers of female patients where likely to report mild burden vs no burden of care. (40,4% females vs 7,7%, p=0,009).
Conclusion: The burden of caregivers is a frequent issue and should be systematically assessed. Older age and need for mechanical ventilation are related with higher severity of burden and should guide clinical suspicion.

P.03: DEVELOPMENT OF A VALID SET OF GUIDELINES AND QUALITY INDICATORS FOR THE CARE OF PATIENTS WITH ALS *Janssens, A.I.W.A.1; Van Damme, P.2,3; Vanhaecht, K.1,4; Hardiman, O.5,6; Sermeus, W.1*

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Amyotrophic Lateral Sclerosis (ALS) is a relentlessly progressive paralyzing disease. Currently, Riluzole is the only drug shown to slow down its progression. However, since this drug affects the course of the survival, rather than the symptoms, the management of ALS mainly consists of symptom control and preserving quality of life. To date, European and American practice guidelines for the management of care of patients with ALS are available. Proper management of ALS has increased substantially since the first publication of these guidelines. Nevertheless, important recommendations remain underutilized and significant variation in the care process and patient outcomes can be identified. Furthermore, the need for supranational efforts to harmonize the current recommendations has been outlined.
The aim of the current study is to develop valid and feasible cross-continental guidelines, quality indicators and evidence-based guidance for decision support of clinicians which can be used to evaluate and ameliorate the quality of care for patients with ALS. This abstract will present the design and methods of the study.
First, a literature review was conducted. Literature was searched for guidelines, quality indicators and evidence-based guidance for decision support of clinicians. Six guidelines, one set of quality indicators and three papers providing aggregated evidence-based guidance for decision support of clinicians were retrieved. Methodological quality was evaluated and a content analysis was performed to identify relevant clinical activities (recommendations), outcomes and baseline variables used in the care for patients with ALS. Secondly, two international delphi surveys are in progress; one involving multidisciplinary 'ALS-care' experts, another involving patients with ALS and their caregivers. The aim of these delphi surveys is to make a selection of clinical activities, outcomes and baseline variables most relevant in the care for patients with ALS, from the perspective of the expert, caregiver and the patient. Finally, these data will be compiled and translated in quality indicators which can be used in the daily care of patients with ALS.

P.04: A FURTHER RASCH STUDY CONFIRMS THAT ALSFRS-R DOES NOT CONFORM TO FUNDAMENTAL MEASUREMENT REQUIREMENT

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Objective: To verify and expand previous evidence of psychometric inadequacies in the ALSFRS-R, in a different sample of subjects suffering from ALS.
Methods: Since 2009, a prospective registry records all incident cases of ALS in Emilia Romagna Region, Italy (4.4 million inhabitants) referred to its 17 neurological departments. For each patient, demographic and clinical information is collected by the physician in charge, including compilation of the ALSFRS-R at each clinical follow-up.
Results: A confirmatory factor analysis on the three-factor model previously found (bulbar, motor, respiratory function) showed a good fit. Rasch analysis on the whole scale showed

the need to collapse some rating categories, confirmed the multidimensionality of the ALSFRS-R, and demonstrated the presence of differential item functioning between patients with spinal vs. bulbar onset. Moreover, some items included in the three ALSFRS-R subscales showed a problematic fit to the respective construct they were intended to measure. Conclusions: The interpretation of a total raw score of ALSFRS-R is hampered by ambiguities due to the different metric properties of the three domains the scale aggregates, and their content and structure. This study confirms that a refinement of ALSFRS-R is warranted, pointing to the need to revise its whole structure, and providing detailed guidelines for its revision.

P.05: SCINTIGRAPHIC EVALUATION OF MILD TO MODERATE DYSPHAGIA IN MOTOR NEURON DISEASE *Katarzyna Szacka1, Anna Potulska-Chromik1, Katarzyna Fronczewska-Wieniawska2, SpychaŁa2, Leszek Królicki2, Magdalena Kuzma-Kozakiewicz1, 3*

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Approximately 30% of patients with motor neuron disease (MND) present swallowing difficulties even in early disease stages. The study objective was to examine the usefulness of esophageal scintigraphy in detecting early stage of dysphagia in MND. Methods: esophageal scintigraphy (ES) including mean transit time (MTT) estimation was performed in 121 MND patients presenting various level of upper (UMN) and lower motor neuron (LMN) involvement. Results: ES was able to detect dysphagia in 81% of MND patients who referred swallowing difficulties and 67% of MND individuals without swallowing complains (subclinical dysphagia). The latter group was characterized by a more benign disease course and a higher percentage of male patients. In MND patients with ES-confirmed dysphagia, the MTT was increased approximately two-fold without significant differences between the clinical phenotypes. The esophageal passage in MND did not depend on age, sex, disease duration and diagnosis delay. The MTT was significantly increased in patients with bulbar-pseudobulbar syndrome as compared to isolated pseudobulbar syndrome what indicates a higher involvement of the LMN deficiency in developing dysphagia in MND. Conclusion: Esophageal scintigraphy is a helpful screening tool in determining early swallowing impairment in patients with MND of various clinical phenotypes.

P.06: QUALIFICATION OF ALS PATIENTS TO LONGITUDINAL HOME REHABILITATION PROGRAM IN POLAND *Jan Sznajder 1,2, Malgorzata Gawel2,3, Magdalena Kuzma-Kozakiewicz 2,3*

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No specific rehabilitation guidelines for ALS patients have been developed to date. It may depend on difficulties carrying out studies of rehabilitation techniques in this group of patients.

The aim of the study was to analyze limitations of ALS patients' qualification to longitudinal studies addressing the efficiency of various rehabilitation techniques in a reference center for neuromuscular diseases in Poland.
Material and methods: 82 ALS patients were admitted to our center in 2013-2015 for diagnostic reasons. Patients were characterized demographically, clinically (including total MRC and ALS-FRSR) and electrophysiologically (MUNIX). All patients underwent physical therapy during hospitalization and were proposed to take part in the rehabilitation program, which involved daily self-performed low intensity exercises (at home) with their periodical adaptation and efficacy analysis every three months (in the center).
Results: Only 20.7% of patients were eligible for entering the rehabilitation program. The remaining 79.3% were not due to a distant residence place (69%) and/or the functional state preventing from travelling (29%). All patients who started home rehabilitation completed the first control visit at the center 3 months after qualification, 41% the second, 29% the third, 12% the fourth and only 6% also the fifth visit (15 months). The reason for the withdrawal from the rehabilitation program was the decrease of the functional status (87%) or death (13%). Among patients who continued exercising and monitoring the motor and functional parameters, there was a significant correlation between the ALS-FRSR, total MRC and the MUNIX results. The latter was found most sensitive in apperceiving minimal functional changes as compared to the two previous methods.
Conclusions: Carrying out longitudinal studies on efficiency of rehabilitation techniques in patients with ALS is highly limited by the diseases progression. Home assessment of the efficacy of the rehabilitation method should be introduced to assure optimal cooperation with ALS patients.

P.07: FROM EXTRA-MOTOR TO EXTRA-EXECUTIVE DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS: A STUDY IN GREECE *Foteini Christidi (1), Ioannis Zalonis (1), Michalis Rentzos (1), Panagiotis Ferentinos (2), Efstratios Karavasilis (3), Vasiliki Zouvelou (1), Theodoros Alexakis (1), Nikolaos Karandreas (1), Ioannis Evdokimidis (1)*

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Background: Executive dysfunction is well documented in amyotrophic lateral sclerosis (ALS). However, other cognitive domains seem to be affected with or without relation to patients' executive profile. The spectrum of cognitive changes in non-demented patients is further supported by widespread structural abnormalities detected through advanced neuroimaging techniques.
Objectives: To investigate cognition beyond the classic executive functions in patients with ALS in Greece.
Methods: Between 2010-2014, we recruited 120 patients diagnosed with ALS according to the revised El-Escorial criteria. Eighty-two patients underwent neuropsychological examination covering processing rate, executive functions (i.e. cognitive flexibility, inhibition control, verbal fluency, problem solving), memory (list learning, story recall, visual recall),

verbal and visual reasoning, visuospatial abilities, expressive language. Depression and pseudobulbar affect were also assessed. Cognitive impairment was defined as score below the 5th percentile based on normative data. Fifty patients also underwent diffusion tensor imaging (DTI) at 3T enabling tractography of the corpus callosum (CC) (i.e. the major white matter tract which is also affected in ALS) and were compared with 14 healthy controls.
Results: Out of 82 patients with ALS, 18 were excluded due to comorbid factors (n=5) and dementia (n=13). Twenty (31.2%) patients were cognitively normal. Out of 64 examined patients, 18 (28.1%) patients showed only executive impairment, 12 (18.8%) patients show executive-plus impairment and 14 (21.9%) patients showed non-executive impairment. Nine (64.3%) out of the 14 non-executive impaired patients had only memory impairment. With regards to CC involvement, patients showed increased diffusivity indices ($p<0.001$) compared to controls.
Conclusions: More than 60% of Greek patients with ALS show cognitive impairment. Even though executive functions are most commonly affected, non-executive impairment, mostly memory, is detected. The contribution of structural brain changes to the observed executive and extra-executive profile and the clinical significance for disease progression is warranted in future studies.

P.08: SEQUENTIAL DECLINE OF EYE MOVEMENT CONTROL IN AMYOTROPHIC LATERAL SCLEROSIS

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Objective: To examine whether impaired oculomotor control in patients with amyotrophic lateral sclerosis (ALS) permits to establish a staging scheme of eye movement control-associated pathology which is in agreement with the model of sequential corticofugal axonal spread of pTDP-43 pathology (Brettschneider et al., Ann Neurol 2013). The human oculomotor system incorporates large parts of the brain including higher function networks as well as brainstem oculomotor circuitry. Hence it provides unique insights into complex brain network pathology.
Methods: Using state-of-the-art video-oculography (EyeSeeCam®), eye movements together with clinical (ALS-FRS) and neuropsychological scores (ECAS) were obtained from 68 ALS patients and 31 matched healthy controls. Additionally, the ALS functional rating scale (ALS-FRS) and the Edinburgh cognitive and behavioural ALS screen (ECAS) were conducted for correlation analysis.
Results: Executive oculomotor deficits as measures with the rate of anti-saccade errors or the frequency of involuntary distracting eye movements (saccadic intrusions) appear to be the first manifestation of impaired eye movement control in ALS (stage 1). Gradually worsening of executive functions accompany the development of gaze-palsy or catch-up saccades that interrupt smooth pursuit eye movements (stage 2). The sequential

appearance of oculomotor dysfunctions were positively correlated (p<0.001) with both, the ALS-FRS, and the ECAS total score.
Conclusion: Our purposed two-staged model indicates that disturbances of eye movement control in ALS develop in a sequential manner, beginning with exclusively executive deficits followed by impaired infratentorial eye movement control pathways such as involved oculomotor nuclei and the brainstem circuitry for saccade generation. This model is an agreement with the neuropathological model of a sequential axonal spread of ALS-related pathology. Hence, investigating of eye movement control in ALS may serve as a technical marker of neuropathological progression.

P.09: GASTROSTOMY IN PATIENTS WITH MOTOR NEURONE DISEASE: A PROSPECTIVE COHORT STUDY (PROGAS)

Dr. Christopher McDermot (on behalf of the ProGas study Group) Reader in Neurology, Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK

Introduction: Gastrostomy feeding is commonly used to support MND patients with severe dysphagia. Although recommended by both the American Academy of Neurologists and European Federation of Neurological Societies, there is currently limited evidence to indicate the optimal method of, and timing for, gastrostomy insertion.
Objective: To identify the optimal gastrostomy timing and insertion method in terms of safety and clinical outcomes.
Methods: This is the first large-scale multi-centre and multi-time point prospective cohort study of patients undergoing percutaneous endoscopic gastrostomy (PEG), radiologically-inserted gastrostomy (RIG) or per-oral image-guided gastrostomy (PIG) in 24 MND Care Centres/Clinics in the UK. Assessments included weight, measures of respiratory function, indices of disease progression and gastrostomy-related data.
Results: 330 patients underwent gastrostomy. 5/163 (3·1%) PEG, 4/121 (3.3%) RIG and 3/43 (7%) PIG patients died within the first 30 days following gastrostomy (p=0.5). Survival following gastrostomy insertion was influenced by the age at onset of MND (p=0.013) and the percentage of weight difference at gastrostomy compared to weight at diagnosis (p=0.001). Peri-procedural patient distress was significantly higher for PEG patients, compared to RIG and PIG patients (p=0.002). Patients who received balloon retention tubes (RIG) experienced a higher rate of complications compared to those who received bumper-retention tubes (PEG and PIG), including displacement (p=0.012); leakage (p=0.001); replacement (p=0.001); and repeated gastrostomy (p=0.001). Following gastrostomy 43 (25.3%) patients gained weight, 43 (25.3%) had stable weight and 84 (48.8%) lost weight. In those who gained weight, these gains were small (median gain=3·1 kg, IR=1.8-6.5).
Conclusions: The three methods of gastrostomy appeared to be as safe as each other from a procedure risk. Survival following gastrostomy appeared to be influenced by disease progression and patient clinical condition at the time of insertion. Percutaneous endoscopic gastrostomy or per-oral image-guided gastrostomy may be optimal as they offer easier post-insertion tube management.

P.10: WHAT IS THE CAUSE OF WEIGHT LOSS 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. Last, weight loss is correlated with survival in patients, 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. Interestingly, pioglitazone, a drug known to increase weight through direct hypothalamic action, did not increase body weight in ALS patients. Mouse models of ALS displayed abnormal food intake behaviors in response to either pioglitazone or fasting. We observed typical ALS-related protein aggregates in the lateral hypothalamic nuclei, but not in other hypothalamic regions, in both patients and mouse models. Consistent with this, only 3 out of 15 tested hypothalamic neuropeptides displayed differential expression in mutant SOD1 mice, and only one was expressed in the lateral hypothalamus. There were decreased numbers of neurons positive for this neuropeptide in mutant SOD1 mice. Last, complementing for the loss of this neuropeptide through intra-cerebro-ventricular delivery with osmotic mini-pumps was able to entirely revert weight loss in mutant SOD1 mice.

In all, our results demonstrate that decreased levels of one single neuropeptide expressed in the lateral hypothalamus accounts for weight loss in ALS, thus providing a pharmacological strategy to treat weight loss in ALS patients.

P.11: SUPPORT NEEDS OF INFORMAL CAREGIVERS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS (ALS) *MSc, Jessica de Wit 1,PhD, Carin Schröder 1, PhD, Anita Beelen 2, PhD, Leonard van den Berg 3, PhD, Anne Visser-Meily 1*

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Introduction: Informal caregivers play a critical role in ALS care and often become the primary caregiver of the patient. ALS caregiving is an intensive task and studies show a

worsening of burden of ALS caregivers during the disease course. Supporting the caregivers with their caregiving tasks may result in increased wellbeing for both caregiver and patient. However, it is still unknown what the specific needs of the caregiver are with regard to support.
Objective: The primary objective of this study was to describe the needs of informal caregivers of patients with ALS regarding support.
Methods: Informal caregivers of patient with ALS were recruited via various ALS teams in the Netherlands. Individual face-to-face semi-structured interviews were conducted with 15 informal caregivers. Caregivers were asked about the care they offered to the patient with ALS. Subsequently, they were asked about their support needs (e.g. need for information or contact with peers) and how they would like to receive support (e.g. face-to-face or via Internet). We transcribed the audio-taped interviews and analysed all data thematically. The Caregiver Strain Index (CSI) was assessed prior to the interview to obtain information about the extent of the caregiver burden. In addition, consent of the patient was asked to gather information from the patient record on the date of birth, date of diagnosis and the score on the ALSFRS of the patient.
Results: Currently, the data is collected. We will present the results of the interviews in terms of needs of support from the perspective of informal caregivers.
Discussion: At present, there are no interventions specifically aimed at supporting informal caregivers of people with ALS with their caregiving tasks. Mapping the needs of the caregivers is a first step towards the development of an intervention.

P.12: END-OF-LIFE PRACTICE IN GERMANY COMPARED TO OTHER EUROPEAN COUNTRIES

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The end-of-life practices in Europe vary greatly. In Germany, legal issues concerning these have been recently on public debate. However, we lack empirical data about current end-of-life practices in Germany in ALS to compare to other European countries.
Medical procedures and causes of death in the terminal phase were retrospectively considered in a sample of N=119 deceased German ALS patients. The physicians involved in the terminal phase anonymously filled out the EURELD questionnaire on medical end-of-life procedures.
No cases of euthanasia and only two physician assisted suicides (PAS) were reported. Withdrawal of life prolonging treatments had led to death in nine cases. 47 patients had received probably death hastening dosage of symptom alleviating drugs, in five cases hastened death being partly the intention. Twelve patients had explicitly expressed a wish for hastened death. In 19 cases, the applied medical procedure had not been discussed with the patient and in seven of those neither with the caregiver.
Results indicate low incidence of purposefully hastened deaths in German ALS patients (1,7%) in the range of suicides in healthy subjects (1,1%) and in French ALS patients (1.3%), but lower than in Swedish (0.3%) and Polish ALS patients. In the Netherlands, where PAS and euthanasia are legal, cases of hastened death are a lot more frequent (20%).
German ALS patients are less approving towards hastened death than the Dutch but more than e.g. Swedish or Polish patients. Legal but also cultural and religious reasons might

account for this discrepancy. Similar to some other European countries with more conservative legal PAS regulations, in Germany administration of intensified drug dosage might be used as an alternative for PAS. Lack of discussion on patient's wish for life termination possibly suggests paternalistic approach in medical counselling in Germany, similar to Sweden but in contrast to the Netherlands.

P.13: EVALUATION OF EXTRAPYRAMIDAL SIGNS IN AMYOTROPHIC LATERAL SCLEROSIS (EXTRALS STUDY) *Andrea Calvo MD PhD, Francesca Dematteis, MD, Stefania Cammarosano MD, Cristina Moglia MD, Carlo A Artusi, MD, Alberto Romagnolo, MD, Serena Angrisano, MD, Andrea Bernardini, MD, Maurizio Zibetti, MD, Leonardo Lopiano, MD, PhD, Adriano Chiò MD, Mario G Rizzone, MD.*

Department of Neuroscience "Rita Levi Montalcini", University of Torino, Torino, Italy

Background: Recent data suggested that ALS may be a manifestation of a spectrum of diseases, with different phenotypic expression including frontotemporal, parkinsonian and psychiatric symptoms.
Objectives: To evaluate the presence of extrapyramidal signs in a prospective series of ALS patients in a longitudinal survey.
Methods: We included 112 consecutive patients with a diagnosis of ALS (July 2012-December 2013) at Torino ALS centre according to El Escorial-Rev criteria(58 M, 35 F; mean age 66 years). To detect the presence of extrapyramidal signs, all patients were evaluated by neurologists expert in movement disorders and scored by MDS-UPDRS. Patients with parkinsonian signs underwent 123I-Ioflupane SPECT, genetic analysis for SNCA, parkin, PINK1, DJ-1, LRRK2 and GBA genes.
Results: At baseline 26 patients (9 classic, 10 bulbar, 4 flail leg, 2 flail arm, 1 upper motor neuron) showed parkinsonian signs (UPDRS I mean score 13.0, UPDRS II mean score 18.3, UPDRS III mean score 39.2). Bradykinesia was present in 100%, rigidity in 78.5%, rest tremor in 18.0%, postural tremor in 35.7%, kinetic tremor in 32.0%, gait disturbances in 82.0% and postural instability in 50.0%. 20 patients underwent 123I-Ioflupane SPECT. Only 3 patients showed a reduction of putaminal dopamine transporter binding. A mutation in LRRK2 gen was found in one patient.
Conclusions: Presence of extrapyramidal signs in ALS patients is a frequent clinical feature. Despite a relevant percentage of new diagnosed ALS patients showed parkinsonian signs (28.0%), a nigrostriatal damage was observed only in a low percentage (13.6%) of patients who underwent 123I-Ioflupane SPECT.

P.14: HEART RATE VARIABILITY IS DECREASED IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS *Tino Prell (1), Thomas Ringer (1), Otto W. Witte (1,2), Julian Grosskreutz (1)*

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Introduction: There is increasing evidence that amyotrophic lateral sclerosis (ALS) is a multisystem disease and that pathological processes extend beyond the motor system.

Because the disease is not curable, patients usually die within 3 years due to respiratory failure. However, also autonomic dysfunction has been reported in ALS, in particular in advanced stages of the disease. Probably cardiovascular dysfunction related to dysautonomia is a cause of sudden cardiac arrest or anoxic encephalopathy after circulatory collapse in these patients.
Methods: We performed a retrospective review of standard polygraphy recordings (Somnoscreen, SOMNOmedics, Randersacker, Germany) of 80 ALS patients. Sleep was staged by personnel blinded to the protocol using standard techniques for scoring sleep stages and arousals. Time-domain parameters of heart rate variability (HRV), such as mean RR interval, SDNN were obtained from nocturnal ECG monitoring. R-R intervals were calculated from artifact and apnea/hypopnea-free periods by the detection of QRS complexes.
Results: HRV variability was found to be reduced in ALS patients. ALS patients are characterized by a marked increase in heart rate.
Conclusion: Our results confirm impaired cardiac autonomic control in ALS. The observed parasympathetic dysfunction and sympathetic predominance could be an explanation of sudden cardiac death.

P.15: META-ANALYSIS, AN OPTIMIZED WAY TO EXPLORE SCIENTIFIC LITERATURE, APPLIED TO MOTOR NEURON DISEASES *Mohamed-Mounir El Mendili (1), Pierre-Francois Pradat (1,2), Salma Masmoudi (3,4)*

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Introduction: Due to a large number of publications, there is a clear need of a pertinent and optimized research tool to integrate multi-scale knowledge related to a specific field of investigation, for instance neurodegenerative diseases. The main objective of the present study is to propose an automated state of art research visual tool using meta-analysis of the scientific literature and to apply this method in the case of spinal muscular atrophy.
Material and methods: Publications search. Publications search was achieved using PubMed platform by specifying keywords related to the field of investigation (spinal muscular atrophy [Title/Abstract]). Research result was saved in xml format (abstracts).
N-grams extraction. Pertinent terms, n-grams, were extracted using Cortext manager platform (2000 n-grams). Co-occurence calculation. Proximity between two or more n-grams was defined as the number of abstracts containing at the same time these n-grams (co-occurence), then computed for all possible n-grams combinations. The user can choose between specific list of n-grams, for instance spinal muscular atrophy, lower motor neurons and neuromuscular junction.
Results: Results can be visualized in several ways: global or restricted graphs, lists of n-grams with co-occurence, and a set of abstracts containing the chosen n-grams by the user.
Conclusion: Our method allows a multi-scale visual representation of the huge body of knowledge generated by the literature in the field of motor neuron diseases. Graph

representation showing the links between pertinent terms may help to generate new scientific hypotheses.

P.16: PILOT ECAS STUDY IN POLISH PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS
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Introduction: The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) has been used as a screening tool in assessment of cognitive dysfunction in patients with ALS. Following the ECAS translation into Polish in 2014 we aimed to asses its utility in examination of the Polish ALS population, as well as to establish the patients specific cognitive and behavioral profile. Material and Methods: 40 patients with ALS diagnosed at the Department of Neurology, Medical University of Warsaw (age 30-75, mean 57.63 ±10.23, 75% males, and 30 healthy controls (age 25-85, mean 50.63 ±18.37), 55.25% males were included into the study. Results: The total score of ALS patients was 95.03 ±21.01, as compared to controls (113.67 ±18.94). ALS patients obtained lower ECAS results in both the ALS-specific domains (67.79±17.79 vs 82.73±15.63): verbal fluency (12.47±7.06 vs 17.6±5.72), and executive functions (30.80±9.89 vs 38.3±8.91) and in the disease non – specific domains: (27.13±5.31 vs 30.93±4.68) with the lowest values in the memory (15.83±4.66 vs 19.33±4.37). Behavioral changes were observed in 20% of patients, the most frequent being apathy (15%), loss of sympathy (10%), diminished interest in others (10%) and loss of social interest (10%). Conclusions: the Polish version of ECAS can be used as a screening tool for cognitive dysfunction in ALS. The cognitive profile of Polish patients with ALS is similar to that achieved in other European populations in the disease-specific domains. The lower results found in the ALS-non-specific domains might be related to a higher mean age in the patients as compared to controls.

THERAPEUTICS

P.17: TIRASEMTIV (CK-2017357) A FAST SKELETAL MUSCLE TROPONIN ACTIVATOR FOR THE POTENTIAL TREATMENT *Shefner JM1, Wolff AA2, Lee J2, Barragan D2, Meng L2, Bian A2, Malik F2, Andrews J2. 1 Barrow Neurological Institute, Pheonix, Arizona, USA 2 Cytokinetics, Inc., South San Francisco, California, USA*

Introduction: Tirasemtiv, a fast skeletal muscle troponin activator, sensitizes the sarcomere to calcium and amplifies the muscle response to submaximal nerve stimulation. It is being developed to improve skeletal muscle function in Amyotrophic Lateral Sclerosis(ALS). Methods: Based on observations from three small phase IIa clinical trials with tirasemtiv, a larger phase IIb clinical trial, BENEFIT-ALS, was conducted in patients with ALS. BENEFIT-ALS(n=711) was an international, randomized, double-blind, placebo-controlled, parallel group study with tirasemtiv administered twice daily at each patient's maximum tolerated dose, up to 500mg daily for 12 weeks.
Results: In all completed studies, tirasemtiv appeared generally safe and well tolerated. In BENEFIT-ALS, there was a statistically significant reduction in the decline of percent

predicted slow vital capacity (SVC) on tirasemtiv (-3.12(±0.90); placebo -8.66(±0.80), p=<0.0001). This difference persisted throughout 28 days after double-blind treatment discontinuation. The percent change from baseline in Muscle Strength Mega-Score also declined more slowly on tirasemtiv (p=0.016 for the difference in slope of decline). A phase III study, CY 4031, was developed to confirm and extend these observations.
CY 4031 is a multi-national, double-blind, randomized, placebo-controlled, stratified, parallel group, study with tirasemtiv treatment up to 52 weeks in patients with ALS. Following completion of two weeks of open-label tirasemtiv (125 mg BID), patients will be randomized 3:2:2:2 to placebo and three different total daily dose levels of tirasemtiv (250, 375 and 500 mg). The primary objective is to assess the effect of tirasemtiv versus placebo on respiratory function in patients with ALS.
Conclusions: The effects on vital capacity and muscle strength observed in BENEFIT-ALS suggest that tirasemtiv has a biological effect. The persistent difference in SVC between treatment groups after double-blind treatment discontinuation suggests that tirasemtiv may have a durable impact. A phase III study, CY 4031, is planned to begin in 2015.

P.18: THE ER-MITOCHONDRIA AXIS AS A NEW THERAPEUTIC TARGET FOR ALS: CHARACTERISATION OF NOVEL DRUG SCREENS *Sarah Müller, Nathalie Welsh, Radu Stoica, Kurt De Vos and Chris C.J. Miller. Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London*

Mitochondria and the endoplasmic reticulum (ER) form close physical associations and these regulate a number of fundamental physiological processes including energy and phospholipid metabolism, Ca2+ homeostasis, mitochondrial biogenesis and transport, ER stress and autophagy. All of these processes are damaged in ALS. Recently, we and others have shown that disruption to ER-mitochondria contacts is a phenotype in several transgenic mouse models of ALS (1,2) This disruption to ER-mitochondria associations can therefore explain many of the seemingly disparate pathological features of ALS. As such, damage to the ER-mitochondria axis represents a new therapeutic target for ALS. Recently, we identified the integral ER protein VAPB and the outer mitochondrial membrane protein PTPIP51 as interacting proteins that function as scaffolding proteins to tether regions of ER with mitochondria. Moreover, we have shown that TDP-43 disrupts these scaffolds (1,3). The VAPB-PTPIP51 interaction thus represents a new therapeutic target for ALS/FTD. Our identification of VAPB and PTPIP51 as ER-mitochondria tethering proteins has enabled us to construct some fast cellular screens for identifying small molecules that might correct defective ER-mitochondria associations in ALS. The first screen involves a mammalian cell GAL4 based 2-hybrid luciferase assay. The second involves use of dimer-dependent EGFPs. Data on the characterisation of these assays and their use to identify molecules that affect VAPB-PTPIP51 and ER-mitochondria associations will be presented.

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P.19: FUMARIC ACID ESTERS INDUCE HYPOXIA-INDUCED FACTOR 1A SIGNALING IN OLIGODENDROCYTE PRECURSOR CELLS *Schmauder K. (1), Wiesner D. (1), Bayer H. (1), Barth E. (1), Ludolph A.C. (1), Witting A. (1)*

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Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease with still few therapeutic options. Recently, a new drug containing dimethylfumarate (BG00012) which belongs to the group of fumaric acid esters (FAEs) has been approved for treating multiple sclerosis. FAEs can induce the transcription factor Nrf2 which leads to neuroprotection via reducing oxidative stress. In addition FAE induce hypoxia-induced factor 1α (HIF-1α) evoking a pseudohypoxic reaction in glia cells. This might be especially relevant in regard to the lactate shuttle between glia cells and neurons. In ALS a dysfunctional lactate shuttle between oligodendrocytes and neurons might be involved in the pathogenesis. Here we investigate if FAEs induce a psydohypoxic response in oligodendrocyte precursor cells (OPCs) related to the release of lactate. We show that FAEs induce HIF-1alpha genes (VEGF; Glut-1) in the OPC cell line OLN-93 as well as in primary OPC from wild type and transgenic mice expressing mSOD1(G93A), which is a well described mouse model of ALS. Reporter assays demonstrated that the FAEs induced VEGF induction was indeed HIF-1α dependent but also Nrf2 dependent. In addition FAEs induced the transcription of the lactate transporter MCT-1 and a release of lactate in primary OPCs, suggesting that FAEs treatment might indeed stimulate the lactate shuttle between oligodendrocytes and neurons. This might be an additional mechanism involved in the therapeutic effect of dimethylfumarate.

P.20: PRECONDITIONING" WITH LATREPIRDINE, AN AMPK ACTIVATOR, DELAYS ALS PROGRESSION IN SOD1G93A MICE *Coughlan KS, Mitchem MR, Hogg MC, Prehn JHM*

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Adenosine 5'-monophosphate-activated protein kinase (AMPK) is a master regulator of energy balance. As energy imbalance is documented as a key pathologic feature of amyotrophic lateral sclerosis (ALS), we investigated AMPK as a pharmacologic target in SOD1G93A mice.
We noted a strong activation of AMPK in lumbar spinal cords of SOD1G93A mice. Pharmacologic activation of AMPK has shown protective effects in neuronal "preconditioning" models. We tested the hypothesis that "preconditioning" with a small molecule activator of AMPK, latrepirdine, exerts beneficial effects on disease progression. SOD1G93A mice (n = 24 animals per group; sex and litter matched) were treated with latrepirdine (1 µg/kg, intraperitoneal) or vehicle from postnatal day 70 to 120.
Treatment with latrepirdine increased AMPK activity in primary mouse motor neuron cultures and in SOD1G93A lumbar spinal cords. Mice "preconditioned" with latrepirdine showed a delayed symptom onset and a significant increase in life span ($p < 0.01$). Our study suggests that "preconditioning" with latrepirdine may represent a possible therapeutic strategy for individuals harboring ALS-associated gene mutations who are at risk for developing ALS.

P.21: TRANSPLANTATION OF A NEURAL STEM CELL SUBPOPULATION AS CELL-BASED THERAPY FOR ALS *Agnese Ramirez, Monica Bucchia, Chiara Simone, Monica Nizzardo, Federica Rizzo, Sara Dametti, Stefania Corti*

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by loss of upper and lower motor neurons in the Central Nervous System (CNS). No effective treatments are presently available for this pathology. We recently showed that transplantation of neural stem cells (NSCs), differentiated from human induced pluripotent stem cells (iPSCs), into animal models of ALS can ameliorate their neurodegenerative phenotype (Nizzardo et al., 2014).
As first, we obtained iPSCs from healthy human skin fibroblasts using a non-viral episomal method. Then, we promoted neuronal fate through an established protocol in order to differentiate iPSCs in NSCs. By FACS selection, we isolated a specific NSCs subpopulation based on positivity for Lewis X+CXCR4+VLA4+. The phenotype of these cells was assessed by morphologic, gene expression, and protein profile analyses.
iPSC-purified NSCs were administered into ALS mice (SOD1G93A mice) by intrathecal injections and neuropathological assays and functional tests were performed to evaluate any modifications of disease hallmarks.
Transplanted Lewis X+CXCR4+VLA4+ NSCs were able to migrate into the CNS and differentiated into the three neuroectodermal lineages after minimally invasive injection, and to engraft into the host spinal cord.
We are currently studying the selection of a specific neuronal subpopulation to improve stem cells survival, migration and engraftment in the CNS, based on their staminality and integrin markers. On this purpose, we are transplanting these selected NSCs in NOD/SCID mice to investigate their engraftment ability. The best selected population will be transplanted in SOD1G93A mice to explore the therapeutic potential of a specific NSC subset as cell-base therapy to treat ALS.

P.22: DHA SUPPLEMENTATION EXTENDS SURVIVAL AND REDUCES INFLAMMATION MARKERS IN MALE ALS MICE *Pascual Torres, Daniel Cacabelos, Victòria Ayala, Jordi Boada, Rosanna Cabré, Alba Naudí, Monica Povedano (1), Reinald Pamplona, Manuel Portero-Otín Department of Experimental Medicine-NUTREN, IRBLLEIDA-University of Lleida, Lleida, Catalonia, Spain.*

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Docosahexaenoic acid (DHA) is an essential fatty acid that plays an important role in central nervous system. DHA regulates neuroinflammation and is decreased in spinal cord of ALS patients, suggesting a role in ALS pathogenesis.
In this work, we attempted to increase DHA content in spinal cord of G93A-hSOD1 mice to restore DHA functions. An isocaloric DHA supplemented diet extended male ALS mice survival, with no effect on female mice survival. Lumbar spinal cord (LSC) fatty acid profiles revealed an increase of DHA levels after the dietary DHA supplementation. Concomitantlly, the DHA supplemented diet decreased LSC levels of arachidonic acid, a precursor of

proinflammatory mediators. A significative negative correlation between DHA and arachidonic acid levels confirmed anti-inflammatory properties of DHA in LSC. On the other hand, oxidative stress is a hallmark of ALS that promotes protein degradation, missfolding and loss of function. In this sense, after dietary DHA supplementation, oxidative damage to LSC proteins was lower than in mice fed with a control diet. DHA reduced DNA oxidation and H2Ax phosphorylation, preventing the DNA Damage Response (DDR) that potentially can lead to death of motor neurons.
In conclusion, we report here gender-specific benefits of a DHA supplemented diet in ALS mice, with neuroprotective effects and modulating neuroinflammation and oxidative stress damage. Collectively, these data suggest a greater benefit of dietary DHA supplementation in male ALS patients.

P.23: TARGETING C9ORF72 EXPANDED RNA G-QUADRUPLEX BY SMALL MOLECULE LEAD COMPOUNDS *Roberto Simone1, Pietro Fratta1, Rubika Balendra1, Stephen Neidle2, Gary Parkinson2 and Adrian Isaacs1*

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Lar 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 formation of nuclear RNA foci and aberrant repeat-associated non-ATG (RAN) translation of dipeptide repeats (DPR). G-rich sequences have a strong propensity to form stable tertiary structures known as G-quadruplexes, known to affect several different aspects of gene expression. We have recently shown that C9ORF72 (GGGGCC)4 repeat can form a very stable RNA G-quadruplex, having profound implications for disease mechanism in ALS/FTD. Screening a highly curated library of small molecules by high-throughput FRET assay for their G-quadruplex-binding properties we found three structurally related lead compounds showing a preferential binding to RNA over DNA G-quadruplex. We characterized their structure- and ion-dependent binding by CD spectroscopy, along with their cell permeability and toxicity. We constructed luciferase-reporter vectors to be able to study quantitatively the effects of small molecules on C9ORF72 RAN translation in 3 different frames (poly-GA, poly-GP, poly-GR) and with different repeat lengths. We will further characterize the effects of those three lead compounds in our recently published Drosophila model of C9-FTD.

EPIDEMIOLOGY

P.24: THE ASSOCIATION BETWEEN NERVOUS SYSTEM DRUGS AND INCIDENCE OF AMYOTROPHIC LATERAL SCLEROSIS *Fabrizio D'Ovidio*, Angelo d'Errico**, Andrea Calvo*, Adriano Chiò* * Department of Neuroscience - University of Turin (Italy) ** Epidemiology Department ASL TO3 – Piedmont Region (Italy)*

No study, to our knowledge, has examined the relationship between prescription of drugs for the nervous system and subsequent onset of amyotrophic lateral sclerosis (ALS).

Therefore, the objective of the present study was to assess this relationship in a large Italian population.
The study population included all subjects identified at the 2001 Italian census, resident in Turin since 1996 (n=737,979). Census data were record-linked to the regional archive of drug prescriptions (active since 1997), and to the ALS Piedmont Registry (active since 1995). In the present study, only new ALS cases diagnosed from 2002 to 2014 were considered. Baseline demographic information was drawn from 2001 census data. Exposure to nervous system drugs (ATC code "N") was measured from 1997 to 2012, or until 1 year before ALS onset. Subjects with at least 5 prescriptions of drugs belonging to the same ATC family were considered ever exposed to a certain family of drugs; in a further analysis the association with cumulative number of prescriptions of each drug family was examined. The association between exposure to nervous system drugs and ALS incidence was evaluated through Poisson multivariate regression models adjusted for sex, age, education and marital status. During the follow-up, 296 subjects in the cohort developed ALS.
The risk of ALS was significantly reduced by exposure to analgesics (IRR=0.32; 95% CI=0.19-0.54) and psychoanaleptics (IRR=0.60; 95% CI=0.39-0.94), deriving in particular from exposure to the sub-families of opioids (IRR=0.12; 95% CI=0.05-0.29) and antidepressants (IRR=0.59; 95% CI=0.37-0.91), respectively. Regarding cumulative dose of drug prescriptions, a significantly decreased risk was found only for opioids (IRR=0.94; 95% CI=0.91-0.97, for a 1-prescription increase).
In conclusion, the study revealed a significant protective role of opioids and antidepressants drugs in developing ALS disease.

P.25: ALS IN SLOVENIA – ANALYSIS OF A PATIENT COHORT AT THE LJUBLJANA ALS CENTRE

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Background: Data on epidemiology and phenotype of ALS are geographically limited and no data have been systematically collected for patients in Slovenia. We performed a retrospective descriptive study on clinical attributes and disease course of patients with ALS treated by a specialised ALS group at the Ljubljana ALS Centre since the foundation of the Centre.
Methods: Data of all 271 patients treated at the Ljubljana ALS Centre in the 10-year period between 2003 and 2012 were analysed: basic demographic characteristics, phenotype of disease onset, diagnostic delay, survival, family history, use of percutaneous gastrostomy (PEG), of non-invasive ventilation and riluzole treatment.
Results: Mean age at symptoms onset was 62.7 ± 11.4 years, median diagnostic delay 11 (IQ range 7–19) months and mean survival from time of enrolment 16.4 ± 15.1 months. 179 (66.1%) patients had spinal onset disease and 71 (26.2%) bulbar disease onset. Factors associated with longer survival were lower age at enrolment, longer diagnostic delay and use of PEG. The proportion of patients using non-invasive ventilatory support was rising through the analysed years.

Conclusions: Disease characteristics and survival in our series are similar to data from other tertiary care centres. The need for non-invasive ventilatory support in ALS patients is increasing.

P.26: BAYESIAN MODELLING OF POTENTIAL ASSOCIATION BETWEEN SOIL MINERAL LEVELS AND SMALL AREA SPATIAL RISK *James Rooney (1), Katy Tobin (1), Mark Heverin (1), Alice Vajda (1), Arlene Crampsie (2), Anthony Staines (3), Orla Hardiman (1,4)*

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3: School of Nursing and Human Sciences, Dublin City University, Dublin, Ireland.
4: Department of Neurology, Beaumont Hospital, Beaumont, Dublin 9, Ireland.

Introduction: We have recently mapped ALS spatial risk in Ireland using Bayesian and cluster analysis methods and electoral division (ED) and small area (SA) levels. Here we extend this analysis to include soil mineral levels from the Irish National Soils Database as Bayesian conditional auto-regression covariates to determine associations with ALS SA risk.
Methods: Data on 45 different soil parameters were obtained under license from National Soils Database (via Irish EPA). We interpolated average values of each soil constituent for each small area using ordinary Kriging. All cases of ALS in Ireland from January 1995 to December 2013 were identified from the Irish ALS register and observed and age & gender standardised expected cases were calculated for each SA.
Besag-York-Mollié (BYM) models were then built including each parameter from the national soils database in turn as a Bayesian covariate in the BYM model. Models were compared using the deviance information criterion (DIC) and separate models were built for ALS subtypes.
Results: 1,701 ALS patients were included - 959 (56%) were male, 938 (55%) had limb onset ALS. 315 Bayesian models were built in total. Of the 315 models built, only one resulted in a coefficient that did not overlap zero. For limb onset cases, total magnesium had a mean coefficient of 0.319 (credible interval 0.033 – 0.607).
Discussion: We report the first spatial analysis of potential association between ALS and soil minerals using a population-based dataset collected over 18 years. Our sole non-zero finding is likely a random finding due to the high number of models built. This is congruent with findings in Guam, which showed that despite high levels of soil aluminium and low levels of calcium in the soil, there was no difference in exposure via food when compared to other locations with lower rates of ALS.

P.27: SURVIVAL ANALYSIS OF SOCIAL DEPRIVATION AND OTHER SPATIALLY STRUCTURED FACTORS IN THE IRISH ALS COHORT *James Rooney(1), Tom Burke(1), M Galvin(1), Katy Tobin(1), Mark Heverin(1), Alice Vajda (1), Marwa Elamin (1), Orla Hardiman(1,2)*

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Introduction: Few studies have examined the role of spatially structured factors in the survival of ALS and while social deprivation has been reported to be an important survival factor cancer and other diseases, areal social deprivation has not been examined as a prognostic factor in ALS previously to our knowledge.
Methods: Spatial variables included population density of the local small area, distance from the coast, distance from Beaumont Hospital (the location of the multidisciplinary clinic) and social deprivation. Population density and social deprivation data was obtained from the pobal HP social deprivation index for 2006 at the 'small area' geometry. Multivariable Roytson-Parmar flexible parametric regression with a proportional hazards scale was used to model survival.
Results: 1,234 patients were included after exclusions. After sequential testing of the spatially structured variables only, small area social deprivation was close to statistical significant on likelihood ratio testing (P = 0.06). An areal social deprivation score in the 'Affluent' range was seen to be associated with improved survival (HR = 0.82 95% CI: 0.67 – 1.00).
Discussion: These results offer the first evidence that areal social deprivation score may be an important factor in the survival of ALS patients. Other spatially structured variables proved not to be important to survival contrasting the UK where London residence was found to be a negative prognostic indicator, and coastal residence was found to be a positive prognostic indicator (Keren et al., 2014). Strengths of this study include a large prospective population based cohort, high spatial resolution for spatial variables and the use of Royston-Parmar flexible parametric regression.

P.28: HIGH RESOLUTION BAYESIAN SMOOTHED SPATIAL RISK MAPPING AND CLUSTER ANALYSIS OF ALS IN IRELAND *James Rooney (1), Katy Tobin (1), Mark Heverin (1), Alice Vajda (1), Arlene Crampsie (2), Anthony Staines (3), Orla Hardiman (1,4)*

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4: Department of Neurology, Beaumont Hospital, Beaumont, Dublin 9, Ireland.

Introduction: Previously we mapped Bayesian smoothed ALS risk in Ireland and performed formal cluster analysis at the level of Electoral Divisions (EDs) (n=3,355). Here, we apply the same statistical methods to the Irish ALS cohort using 18,222 small areas (SAs).
Methods: All cases of ALS in Ireland from January 1995 to December 2013 were identified from the Irish ALS register. Census data at SA resolution from the 2006 census were used as an approximate mid-point estimate of population to calculate an average population for the

period, and standardized incidence rates (SIRs) were calculated for the 18,222 SA's. Bayesian conditional auto-regression was applied to produce smoothed relative risks (RR). Cluster analysis was performed using the software SaTScan. In addition, Bayesian and linear regression were used to examine the relationship between population density and RR, and social deprivation and RR.
Results: 1,701 cases were included. Bayesian smoothed maps of relative risk at small area resolution matched closely our previous analysis at ED level. Cluster analysis identified two areas of significant low risk that closely corresponded with those we found at ED level – although the SA level areas were smaller in range. In contrast to our ED level findings, population density was not related to RR. Social deprivation did not explain the pattern of spatial risk for ALS in Ireland.
Discussion: We have replicated our previous results for ED level data at a much higher spatial resolution including 18,222 small areas. Bayesian risk mapping shows a similar overall spatial pattern of risk at both geometries for all cases, males and females. Cluster analysis via SaTScan identified two statistically significant low risk areas in counties Clare and Kilkenny as previously, however the SA geometry allowed us to define these clusters more precisely.

BIOMARKER

P.29: ALTERATION OF KINESINS EXPRESSION INVOLVED IN BIDIRECTIONAL TRANSPORT IN BLOOD OF PATIENTS WITH MND

Magdalena Kuzma-Kozakiewicz1,3, Beata Kazmierczak 2,3, Agnieszka Chudy2, Beata Gajewska2,3, Anna Baranczyk-Kuzma 2,3

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Introduction: Disturbance of intracellular transport are one of the possible mechanisms of motor neuron damage. The aim of the study was to evaluate the expression of kinesins involved in the anterograde (KIF1B, KIF5C) and retrograde (KIFC3) intracellular transport in peripheral blood mononuclear cells (PBMCs) of patients with motor neuron disease (MND).
Material and methods: PBMCs were obtained from 74 MND patients of various clinical phenotypes, 65 blood donors (healthy control I), and 29 cases with other neurological diseases (disease control II) divided into the subgroup IIA (atypical parkinsonism) and the subgroup IIB (ALS mimicking disorders). mRNA expression was studied by real-time qPCR, protein level by Western blotting. Results: In the entire MND group and in classic ALS, the mRNA expression of kinesins KIF5C and KIFC3 but not KIF1B was significantly lower than in the healthy control I. KIF1B-mRNA expression was significantly higher in classic ALS than in PMA and in classic ALS compared to ALS mimicking disorders (the subgroup IIB). In MND the protein level of KIF5C, but not the KIFC3, was lower than in healthy cases.
Conclusions: The changes in expression of kinesins may alter bidirectional intracellular transport in peripheral blood mononuclear cells of MND patients, especially in classic ALS. The level of KIF5C expression might be useful in diagnosis of ALS, while KIF1B expression may help discriminate ALS from ALS mimicking disorders.

P.30: BLOOD-BASED MIRNAS POTENTIAL BIOMARKERS IN MOTOR NEURONE DISEASE
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Sheffield Institute for Translational Neuroscience, Sheffield University, UK

Background: Motor neurone disease (MND) is a fatal neurodegenerative disease leading to paralysis and death. Effective biomarkers would help to diagnose MND, aid in drug development and track disease progression and possible prognosis. microRNAs (miRNAs) are small non-coding RNAs that direct post transcriptional gene regulation. They have been shown to be dysregulated in neurodegenerative diseases including MND and are found at detectable levels in the blood, therefore having the potential to be used as biomarkers of disease. The hypothesis of this study is that candidate circulating miRNAs identified that distinguish MND patients from control cases will change over time with disease progression.
Aims: The objective of this study was to investigate miRNA expression in MND patient serum samples to assess whether;
1. miRNA expression alters in patients taking riluozle or riluzole naïve at time of diagnosis.
2. miRNA expression alters over time with disease progression.
Methods: miRNAs were extracted from patient serum samples at time of diagnosis (or within three months) and a subsequent serum sample was taken at least three months post diagnosis. Qiagen miScript reverse transcription, pre-amplification and qPCR was carried out to investigate the expression of miR-206, miR-143-3p and miR-374b-5p between patients taking riluzole and riluzole naïve and subsequently to establish the miRNA expression over time with disease duration.
Findings: There was no significant difference in the expression of miR-206, miR-143-3p and miR-374b-5p between patients taking riluzole or riluzole naïve patients at time of diagnosis. However, as the disease progressed a significant increase in miR-143-3p was identified in MND patients, while a significant decrease was found in miR-374b-5p.

P.31: MONOCYTE SUBTYPES IN ALS *Lisa Zondler(1), Samira Khalaji(2), Kathrin Müller(1), Corinna Bliederhäuser(1), Veselin Grozdanov(1), Wolfgang P. Ruf(1), Axel Freischmidt(1), Patrick Weydt(1), Albert C. Ludolph(1), Kay-E. Gottschalk(2), Karin M. Danzer(1), Jochen H. Weishaupt(1)*

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2: Ulm University, Department of Experimental Physics.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by progressive loss of motor neurons. Neuronal loss in ALS is accompanied by a neuroinflammatory reaction including recruitment of peripheral monocytes to the CNS. Peripheral human monocytes are dividable into two subpopulations, the pro-inflammatory CD14++ monocytes and the regenerative CD16++ monocyte subtype.
The aim of this study is to decipher the contribution of the different monocyte subpopulations to disease initiation and progression in ALS patients, preclinical ALS mutation carriers and in an ALS mouse model.
By flow cytometry, we found the pro-inflammatory CD14++ monocyte subpopulation to be significantly overrepresented in the blood of ALS patients and pre-symptomatic carriers of ALS mutations compared to healthy, age-matched controls. Functional characterization of

the CD14++ subpopulation revealed impaired phagocytosis and vesicle trafficking in CD14++ monocytes from sALS patients, as well as altered adhesive properties and migration potential. Concordantly, we found CD14++ monocytes of sALS patients to exhibit a distinct gene expression signature, not only separating sALS monocytes from monocytes of healthy donors, but also substantiating the functional differences described above.
Further, we found that immunomodulatory treatment of ALS patient derived CD14++ monocytes in vitro reverses the shift of monocyte subpopulations observed in ALS patients and delays the disease onset in an ALS mouse model in vivo.
Taken together, our findings indicate the involvement of especially the CD14++ monocyte subpopulation in ALS and we present a possible strategy of pharmacological intervention.

P.32: DOES MUNIX METHOD REFLECT CLINICAL DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS? *Malgorzata Gawel MD, PhD, Magdalena Kuzma-Kozakiewicz MD, Ass Prof. Department of Neurology, Medical University of Warsaw, 1A Banacha street, 02-097 Warsaw, Poland, Neurodegenerative Diseases Research Group, Medical University of Warsaw, Warsaw, Poland*

The aim of our study was to assess the value of the MUNIX method in reflecting clinical dysfunction in patients with ALS and to assess intra-rater reproducibility of MUNIX..
Patients and methods: The study population included 15 patients. The mean patient age at the disease onset was 55 years (28-75), the mean duration of the disease was 10 months. MUNIX in abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibial anterior (TA), extensor digitorum brevis (EDB) and abductor hallucis (AH) muscles were estimated. Muscle strength according with Medical Research Council (MRC) and ALS Functional Rating Scale (ALSFRS-R) were assessed. The global MUNIX and MRC score was calculated. In 11 patients the protocol of the study was repeated every 3 months; at least two times (2-5). Results: There was no significant difference between MUNIX test and re-test values in APB, ADM, BB, TA, EDB and TA muscles ($P>0.05$).The most marked variability between test and re-test was in BB muscle (7.53%). There was difference in test-retest In global Munix score ($P=0.02$) but with low variability (1.26%). Global MUNIX values correlated with global MRC scores ($P<0.05$) and MUNIX value correlated with MRC score for each muscle: ABP ($P<0.05$), ADM ($P<0.05$), TA ($P<0.05$), EDB ($P<0.05$), AH ($P<0.05$). Less significant correlation was found between MRC score and MUNIX value in BB muscle ($P<0.1$). There was a significant correlation between MUNIX global score and clinical dysfunction measured by ALSFRS-R scale and ($P<0.05$), but there was no relationships between MUNIX and duration of the disease.
Conclusion: The study confirms that MUNIX method is a sensitive tool reflecting motor dysfunction and could be a good biomarker for ALS progression. However MUNIX seems to be more adequate method for distal muscles and we experienced that its implementation is easier in muscle with more preserved strength.

P.33: THE ALS STRATIFICATION PRIZE- USING BIG DATA AND CROWDSOURCING FOR CATALYZING BREAKTHROUGHS IN ALS *Neta Zach, Robert Kueffner, Hagit Alon, Raquel Norel, Thea Norman, Lara Mangarvite, Orla Hardiman, Nazem Atassi, Merit Cudkowicz, Alexander Sherman, Jason Walker, Ervin Sinani, Igor Katsovskiy, David Schoenfeld, Melanie Leitner, Gustavo Stolovitzky*

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with significant heterogeneity in its progression. This heterogeneity makes research, clinical care and drug development difficult. To overcome this challenge, we developed the Pooled Resource Open-access ALS Clinical Trials (PRO-ACT) platform. The PRO-ACT database, the largest clinical trial database, contains information from 8600 ALS patients and since its launch have attracted requests to use from over 400 researchers.
Prize4Life, in collaboration with DREAM, used the PRO-ACT data to launch the DREAM ALS Prediction Prize4Life Challenge, a crowdsourcing Challenge seeking the development of more accurate tools for estimating disease progression at the individual patient level. The DREAM ALS Prediction Prize4Life drew 1000+ registrants that used three months of clinical data to predict disease progression a year later. In a simulation of a clinical trial, use of the winning algorithms for patient enrolment enabled a trial size reduciton of 20%. The best performing methods also uncovered several novel predictors of disease progressions that can shed light on the mechanisms behind the disease. These algorithms are now being used in several clinical trials and clinics.
These results demonstrate the value of large datasets and crowdsourcing Challenges for developing a better understanding of ALS natural history, prognostic factors and disease variables.
To further the use of Big Data in ALS, we are currently developing another crowdsourcing Challenge, The DREAM ALS Stratification Prize4Life Challenge. The Challenge, collaboration between Prize4Life, DREAM and Sage Bionetworks will invite hundreds of participants from all over the world to identify features that stratify ALS patients into meaningful subgroups for better prediction of their clinical profile and disease course. The challenge will be launched in June 2015.

P.34: THE ROLE OF THE SENSEWEAR DEVICE AND GHRELIN FOR METABOLISM IN AMYOTROPHIC LATERAL SCLEROSIS *Czell D.1,3 Baldinger R. 1,4, Schneider U.1, C. Neuwirth1, Weber M.1,2, MD*

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Background: Metabolism and the nutritional status are very important prognostic factors for survival in patients with amyotrophic lateral sclerosis. However, so far it has been challenging to reliably predict energy expenditure in the disease course. SenseWear® Armband is a good device to accurately measure resting energy expenditure (REE) in healthy subjects. Ghrelin and Leptin which regulate appetite and metabolism were recently found to have a neuroprotective effect in neurodegenerative diseases.
Objective: To evaluate the SenseWear® armband for measuring REE in patients with amyotrophic lateral sclerosis and to measure ghrelin, leptin and determine caloric intake and nutritional status in patients with amyotrophic lateral sclerosis (ALS).
Methods: First EE and coloric intake was measured over 3 day in 10 healthy controls. In two patients and their spouses EE and caloric intake were measured over 48 hours. In addition ghrelin and leptin was measured at an interval of 6 months.
Results: Only 2 patients and their caregivers could be recruited because drawing blood for Leptin and Ghrelin was a significant burden for patients and caregivers during study visits. However, total ghrelin was 30% lower in ALS patients (P1, P2) compared to spouses (C1, C2) and normal values. Comparing EE between consecutive days revealed reliable measurements (P1: 2064 and 2124 kcal/ day, P2: 2020 1969 kcal/ day, C1: 1740 and 1599 kcal / day, C2: 1569 and 1422 kcal / day). Despite balanced EE and caloric intake both patients had profound weight loss.
Conclusion: The SenseWear® device seems to reliably measure EE. Our data suggest that ghrelin plays a key role for the nutritional status in ALS.

P.35: RETINAL INVOLVEMENT IN ALS: A STUDY WITH OPTICAL COHERENCE TOMOGRAPHY AND DIFFUSION TENSOR IMAGING *Annemarie Hübers1, Kathrin Böhm1, Jens Dreyhaupt2, Hayrettin Tumani1, Jan Kassubek1, Hans-Peter Müller 1, Albert C. Ludolph1, Elmar H. Pinkhardt1*

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Background: Retinal thinning has been reported in a variety of neurodegenerative conditions, and a recent study reported thinning within the inner nuclear layer (INL) in two patients with C9orf72 mutations. Here, we aimed to study retinal alterations in ALS. We hypothesize that changes of retinal layers, as measured by Optical Coherence Tomography (OCT) may reflect overall neurodegeneration. If so, then retinal involvement may be regarded as a possible biomarker.

Methods: Spectral domain OCT (Heidelberg Spectralis HD OCT) images were analized with an in house developed semiautomatic algorithm [Schneider et al, Journal of neural transmission, 2013] to calculate the thickness of retinal layers in 73 ALS patients (51 spinal, 22 bulbar onset) and 20 matched controls. In a subgroup of 30 patients, Diffusion Tensor Imaging (DTI) data was acquired and the region of interest (ROI) based fractional anisotropy (FA) was measured in the corticospinal tract (CST). Demographic data and ALS-FRS score were collected for correlation analysis.
Results: We saw a significant thinning of the INL (p=0.04) and the retinal nerve fibre layer (RNFL) (p=0.004) in patients. Yet, subgroup analysis showed a significant difference only in spinal onset patients (INL: p=0.006; RNFL: p=0.002). Patients showed significantly reduced FA values of the CST compared to controls (p<0.001). We found a significant inverse correlation between whole retinal thickness and FA values of the CST in spinal onset patients (p=0.005) and controls (p=0.001).
Conclusions: Our study provides evidence for a retinal involvement in ALS. Also, we saw a significant inverse correlation between retinal thickness and FA of the CST in spinal onset patients and in controls. Interestingly, while ALS patients show a reduction in FA and retinal thickness, the link between both features remains consistent. We conclude that retinal involvement is in fact associated with neurodegeneration and may be regarded as a potential biomarker.

P.36: POST MORTEM MRI TO DECIPHER CORPUS CALLOSUM INVOLVEMENT IN ALS

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Background: In vivo magnetic resonance diffusion tensor imaging studies have demonstrated reduced fractional anisotropy and increased radial diffusivity, particularly in the body of the corpus callosum (CC) in ALS patients, where interhemispheric fibers connect the two primary motor cortices (M1).
Objectives: The aim of this study was to explore the connectivity of the CC in a post mortem human brain, in relation to the topographical body representations of the motor homunculus in M1.
Methods: Ultra-high-resolution structural and diffusion-weighted steady-state free precession (DW-SSFP) magnetic resonance images of a post mortem control brain were acquired at 7 Tesla. Hand, foot and face areas in M1 were identified, guided by a set of reference masks in standard Montreal Neurological Institute space and non-linearly registered to the post-mortem structural scan. Subsequently, these regions-of-interests were adjusted to match the individual gyrification pattern and used as seed masks for probabilistic tractography (as implemented in FSL).
Results: It was possible to separate the callosal motor fibers (CMFs) according to different body representations in the motor homunculus.
Conclusions: Future research will include investigation of the CMFs in post-mortem ALS brains, in order to 1) study differences in DTI metrics between ALS patients and healthy

controls in different parts of the corpus callosum in relation to clinical phenotype and 2) enable correlation of ex vivo MRI measures with neuropathological markers derived from the same tissue.

P.37: ELEVATED INFLAMMATORY PARAMETERS IN WOMEN WITH PROGRESSIVE BULBAR PALSY *Beata Chelstowska 1, Agata Majewska 1, Magdalena Kuzma-Kozakiewicz 2,3*

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Introduction: Although the role of inflammation in development of motor neuron disease (MND) is still unclear, there is abundant evidence of increased concentrations of selected factors involved in the promotion of inflammatory processes in the patients'tissues and body fluids.
Aims: The aim of the study was to analyze the level of basic inflammation parameters (IP) in blood of MND patients with various clinical phenotypes.
Material and methods: The study included 285 MND Polish patients (134 females and 151 males, mean age 56.7 years) of Caucasian origin. The analyzed clinical parameters included diagnosis delay, disease duration, site of onset (bulbar/limb), phenotype (classic; progressive muscular atrophy, PMA; progressive bulbar palsy, PBP; primary lateral sclerosis, PLS; flail arm/flail leg syndrome, FA/FL), functional status (ALS-FRS), and comorbidities. Laboratory parameters included C Reactive Protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell count (WBC) and fibrinogen concentration (FC).
Results: WBC was normal in all patients. Elevated CRP was found in 8%, ESR in 11% and FC in 12% of all patients. CRP, ESR, FC were more frequently increased in women compared to men ($p<0.001$), in PBP compared to classic ALS ($p<0.05$) and in bulbar compared to limb onset ($p<0.02$). Although the females were significantly more numerous in the PBP and bulbar onset groups, the highest level of analyzed parameters (mean CRP= 11.2 mg/L ; ESR= 18 mm/h; FC= 412.4 mg/dL) were observed in women with PBP, but not in women with other clinical phenotypes. The patients age, diagnosis delay, ALS-FRS, disease duration, and comorbidities correlated neither with the frequency of elevated inflammatory parameters nor their level.
Conclusion: The inflammatory parameters are increased in approximately 10% of MND patients independently of the disease stage. Elevated inflammatory parameters are more frequent in women with progressive bulbar palsy compared to other MND phenotypes.

P.38: INCREASED RESTING STATE FUNCTIONAL CONNECTIVITY IN PRE-SYMPTOMATIC ALS *Menke RAL (1), Proudfoot M (1,2), Wuu J (3), Andersen PM (4), Talbot K (1), Benatar M (3), Turner MR (1)*

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4: Department of Clinical Neuroscience, Ume? University, Sweden.

Background: The pathology of amyotrophic lateral sclerosis (ALS) is believed to begin significantly before the onset of symptoms. Advanced MRI applied to pre-symptomatic

carriers of highly penetrant genetic mutations linked to the development of ALS offers a unique tool for the investigation of structural and functional brain changes in advance of clinically manifest disease.
Methods: T1-weighted, diffusion-weighted, and resting-state functional MRI (rs-fMRI) data were acquired in 12 pre-symptomatic ALS gene carriers (psALS; 10 SOD1 and 2 C9ORF72), 12 age-matched controls and 12 age-matched ALS patients. Group comparisons were performed for measures of cortical thickness, grey matter density, shape and volume of sub-cortical structures, and metrics derived from the diffusion tensor. Resting-state functional connectivity (FC) abnormalities for the psALS group were investigated within networks that differed between affected patients and controls.
Results: Compared to controls, significant grey matter atrophy was observed in the affected ALS patients in the right primary motor cortex and right caudate; no grey matter atrophy or markers of reduced white matter integrity were detected in the psALS group.
Compared to controls, higher FC was seen in the affected ALS patients within the sensorimotor network and between a network comprising precuneus, cingulate and middle frontal regions and the cerebellum. While sensorimotor network FC was similar in psALS and controls, FC of the precuneus-cingulate-middle frontal network with the cerebellum was significantly higher in psALS compared to controls, similar to that observed in the affected patient group.
Conclusions: Functional connectivity changes may be among the earliest non-invasively detectable brain abnormalities in pre-symptomatic carriers of gene mutations linked to ALS. With replication and refinement, functional MRI might hold potential as a harmacodynamics biomarker relevant to the development of neuroprotective therapies.

P.39: QUANTIFYING SPINAL CORD ATROPHY: A MRI TOOL TO INVESTIGATE THE SELECTIVITY OF MUSCLE WEAKNESS IN SMA *Mohamed-Mounir El Mendili (1), Timothee Lenglet (2,3), Maria del Mar Amador (4), Stephane Lehericy (5,6), Habib Benali (1), Pierre-Francois Pradat (1,7)*

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7: Departement des Maladies du Systeme Nerveux, Pitie Salpetriere Hospital, Paris, France.

Introduction: Spinal muscular atrophy (SMA) is characterized by lower motor neuron (LMN) loss that leads to proximal muscle wasting and paralysis. In this study, we investigated the link between spinal cord atrophy profile and the proximal muscles deficits in spinal muscular atrophy.
Material and methods: Patients with type III/V SMN1-linked SMA and age-matched controls were recruited. Patients were scored using manual muscle testing (MMT). Subjects were scanned at 3T MRI system (Tim Trio, Siemens Healthcare). Spinal cord was imaged using a

T2-weighted sequence (52 sagittal slices, FOV=280mm, TE/TR=1500/120ms, voxel size = 0.9x0.9x0.9mm3). Data were segmented. Cord cross-sectional (CSA) area was computed along the cervical spinal cord. The difference in CSA between SMA patients and controls was assessed using permutation test (one side). Spearman's rank correlation coefficient was used to investigate correlations between CSA and MMT. A supra-significance level alpha=10-3 was used.
Results: CSA showed a significant atrophy gradient mainly located between C3 and C6 vertebral levels with a cord atrophy rate ranging from 5.4% to 23%. There were no correlations between CSA and MMT nor between CSA and disease duration.
Conclusions: Atrophy predominates in the spinal segments innervating the proximal muscles. The missing correlations between CSA and MMT as well as the clear distal deficit showed by MMT in our SMA population; suggest that the loss of motor neuron cell bodies may not recapitulate all the mechanisms responsible for clinical deficits.

P.40: CALCIUM DISTURBANCE IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH ALS

Jingyu Liu, Tino Prell, Ayse Malci, Vedrana Tadic, Otto W. Witte, Julian Grosskreutz

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Background: Amyotrophic lateral sclerosis (ALS) is a severe progressive neurodegenerative disease which lacks adequate diagnostic and longitudinal disease markers. Brain and spinal cord biopsy are not a clinically feasible. Peripheral blood mononuclear cells which are involved in numerous diseases may be of high value for neurodegenerative and neuroinflammatory events. Ca2+ dysregulation plays a central role in the pathophysiology of ALS which is now thought to be a system disease; thus studying calcium homeostasis i.e. after stimulating p2x receptors in PBMC may help to identify disease specific signatures.
Objectives: We attempted to determine (i) P2X4R and P2X7R expression, and (ii) function, induced by direct p2x4 and indirect P2X7 receptor stimulation, as determined by cytosolic free Ca2+ influxes measurements and (iii) elucidate the role of P2XR activation involvement in pathophysiological alterations of mononuclear blood cells from ALS patients.
Methods: We used peripheral mononuclear blood cells and isolated monocytes derived from ALS patients and healthy controls. Cells were stimulated by exogenous ATP, an inflammatory stimulus (lipopolysaccharide, LPS) and agonist for glutamate receptor (kainate). Calcium transients in individual monocyte were recorded by FURA-2 calcium imaging. P2XR expressions were measured by immunhistochemistry and western blot.
Result: 81.5% monocytes in ALS patients and 78% in healthy controls responded to ATP. The amount of Ca2+ influxes, induced by the P2XR agonist ATP 10uM, were higher in controls than in patients at second and third stimulation ($p<0.05$). Moreover, no responses were recorded after stimulation with kainate and other LGIC activators, excluding other calcium sources in the response.
Conclusion: Our results provide evidence that in monocytes from ALS patients function of the purinergic P2X receptor are decreased with respect to healthy controls. This approach may helpr to understand ALS pathophysiology on a systems level and contribute as non-invasive surrogate marker for clinical trials in ALS.

P.41: POLYUNSATURATED FATTY ACID COMPOSITION OF BLOOD LIPIDS AS A POTENTIAL BIOMARKER FOR ALS PATIENTS *L. Robelin1,2, A. Henriques1,2, H. Blasco3,4, M.C. Fleury5, P. Corcia3,6, A. Echaniz-Laguna1,2,5, T. Lequeu1,2, G. Rudolf5, M. Bergaentzle7, 8, C.R. Andres3,4, C. Tranchant5, J.P. Loeffler1,2, E. Marchioni7, 8 and J.L. Gonzalez De Aguilar1,2*

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6: CHRU de Tours, Centre SLA, Tours.
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8: CNRS, UMR 7178, Strasbourg, France.

Beyond motor neuron pathology, ALS presents with systemic alterations of lipid metabolism that appear to contribute to the clinical course of the disease. The variations in the composition of polyunsaturated fatty acids (PUFA) in blood lipids are considered as an index of altered lipid metabolism in conditions such as cancer and diabetes. In this study, we analyzed PUFA composition in blood lipids to identify markers of diagnosis and prognosis. We enrolled 117 ALS patients. Forty-eight were age and gender matched with control subjects. We extracted total lipids from serum and blood clot, and separated derived fatty acid methyl esters by gas chromatography. In general, proportions of several members of the PUFA ω3 and ω6 series showed significant differences between ALS patients and healthy subjects, in particular in blood cell pellets. ROC analysis revealed that docosapentaenoic acid (DPA, 22:5 n-3) in blood cell pellets was able to distinguish those individuals with the disease from those without the disease (AUC= 0.86, P<0.0001). In serum, DPA levels correlated with disease status, as estimated by the ALSFRS-R score. Multivariate Cox analysis, with age, BMI, site of onset and ALSFRS-R as covariables, showed that serum DPA was associated with better survival rates (HR=1.4, 95%CI=0.02-0.79, P=0.0238). Our findings therefore deserve further validation in larger cohorts for being used to assess disease outcome and effects of disease-modifying drugs.

IMAGING

P.42: UNRAVELLING THE MOLECULAR MECHANISMS BEHIND CORTICOSPINAL MOTOR NEURON DEGENERATION IN ALS *Christine Marques1, 2 and Caroline Rouaux1, 2*

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2: Université de Strasbourg, Faculté de Médecine, Strasbourg, France.

Amyotrophic Lateral Sclerosis (ALS) is the most common neurodegenerative disease of the adult motor system. It leads to progressive muscle paralysis and death within 5 years, and remains incurable. At the cellular level, ALS is characterized by the combined degeneration of two neuronal populations: the corticospinal motor neurons (CSMN), and the spinal motor

neurons (SMN). Despite this precise clinical description, the role of CSMN and more broadly that of the cerebral cortex, has never been directly interrogated so far, and is still poorly understood.
My PhD project aims at better characterizing the cortical pathology in ALS, and at deciphering the molecular mechanisms that selectively trigger CSMN degeneration during the course of the disease. My hypothesis is that CSMN dysfunction and degeneration centrally contribute to ALS onset and progression.
In order to characterize the cortical pathology in ALS, I have performed molecular and histological analyses in wild type and Sod1G86R mice, a rodent model of ALS. My preliminary data show that CSMN progressively degenerate in Sod1G86R mice. Interestingly, in drastic contrast to the well-described spinal pathology, we observed that CSMN degeneration takes place in absence of major glial pathology. In addition, I develop an approach that combines retrograde labeling from the spinal cord and purification of retrogradelly labeled CSMN via fluorescent activated cell sorting (FACS) from wild type and Sod1G86R mice, in order to perform RNAseq analyses. These transcriptomic analyses of pure CSMN populations, collected at different stages of the disease, will enable us, for the first time, to unravel the molecular mechanisms behind CSMN dysfunction and degeneration in an ALS mouse model.
On the long run, this project is intended to identify new molecular pathways that may inform the development of alternative therapeutic strategies for ALS and related CSMN-specific neurodegenerative diseases.

POSTER SESSION II
Friday 22nd May, 17.30-19.00 (Chairs:)

GENETICS

P.43: ASSOCIATION OF GSTP1 POLYMORPHISM WITH GLUTATHIONE S-TRANSFERASE AND PEROXIDASE ACTIVITY IN MND *Beata Gajewska1,2, Beata Kazmierczak1,2, Magdalena Kuzma-Kozakiewicz 2,3, Anna Baranczyk-Kuzma 1,2*

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Glutathione S-transferase pi (GSTP1) is a crucial enzyme in detoxification of electrophilic compounds and organic peroxides. Together with Se-dependent glutathione peroxidase (Se-GSHPx) it protects cells against oxidative stress which may be a primary factor implicated in motor neuron disease (MND) pathogenesis. We investigated GSTP1 polymorphisms and their relationship with GST and Se-GSTPx activities in a cohort of Polish patients with MND. Results were correlated with clinical phenotypes. The frequency of genetic variants for GSTP1 exon 5 (I105V) and exon 6 (A114V) was studied in 104 patients and 100 healthy controls using real-time PCR. GST transferase activity was determined in serum with 1-chloro-2,4-dinitrobenzene, its peroxidase activity with cumene hydroperoxide, and Se-GSHPx activity with hydrogen peroxide. There were no differences in the prevalence of GSTP1 polymorphism I105V and A114V between MND and controls, however the

occurrence of CT variant in codon 114 was associated with a higher risk for MND. GSTP1 polymorphisms were less frequent in classic ALS than in progressive bulbar palsy (PBP). In classic ALS C* allelic variant all studied activities were significantly lower than in classic ALS A*. GST peroxidase activity and Se-GSHPx activity were lower in classic ALS C* than in control C*, but in classic ALS A* Se-GSHPx activity was significantly higher than in control A*. It can be concluded that the presence of GSTP1 A114V but not I105V variant increases the risk of MND, and double GSTP1 polymorphism may result in lower protection of MND patients against the toxicity of electrophilic compounds, organic and inorganic hydroperoxides.

P. 44: TURKISH ALS CASES WITH THE C9ORF72 EXPANSION: A GENOMIC AND METHYLOMIC APPROACH *Ceren Iskender, Hamid Hamzeiy, Fulya Akcimen, A. Nazlı Başak*

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Each genetic discovery broadens the phenotype associated with the clinical definition of amyotrophic lateral sclerosis (ALS). C9orf72, as the leading cause of familial and sporadic ALS, is associated with a highly variable clinical course ranging from fatal motor neuron disease to a slowly progressive form of frontotemporal dementia (FTD); gathering ALS, FTD and Alzheimer's disease under the same roof. Considering this phenotypic diversity, identifying correlations between disease duration, age of onset, site of onset and disease subtype will help targeting disease modifiers and eventually understand the underlying mechanisms. Several studies show that hypermethylation is a common phenomenon for repeat expansion disorders like FRDA, Fragile X and DM acting as an epigenetic disease modifier. It has been recently suggested that both C9orf72 promoter hypermethylation and histone trimethylation is associated with C9orf72-based ALS and FTD. Reduced expression levels observed in case of hypermethylation may lower toxic C9orf72 RNA accumulation and thus can explain the prolonged disease duration reported in some patients. In this study we are embracing a combined genetic and epigenetic approach to investigate 36 Turkish patients carrying the C9orf72 expansion. Among these patients, 28 present with ALS, five with ALS/FTD and two with FTD along with a yet-asymptomatic carrier. Additional clinical differences apply in case of the ages/sites of onset and disease durations; also, intrafamilial phenotypic heterogeneity is observed in two families. We are performing exome sequencing to see the genetic backgrounds of the mutation carriers. Furthermore, restriction enzyme digestion and bisulfite sequencing are used to observe the promoter methylation status followed by qPCR to understand the effects of hypermethylation on C9orf72 mRNA levels, both in patients with or without the expansion and in controls. With this approach we aim to conduct a comprehensive study to understand the functional consequence of the C9orf72 repeat expansion by identifying disease modifiers.

P.45: ALS-ASSOCIATED GENES AS BLOOD RNA BIOMARKERS OF SPINOCEREBELLAR ATAXIA TYPE 2 *Nesli Ece Sen1, Suna Lahut1, Gulden Akdal2, Halil Gulluoglu3, A. Nazli Basak1*, Georg Auburger4**

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Spinocerebellar Ataxia Type 2 (SCA2) is an autosomal dominant movement disorder with death of Purkinje neurons in the cerebellum and of motor neurons in the spinal cord and cortex. Expansions in the poly-glutamine domain of ATXN2 beyond a size of 33 units constitute the genetic cause of SCA2. Interestingly, intermediate length expansions (27-33 units) are associated with an increased risk for ALS. While its function is not fully understood, it is known that ATXN2 binds to the 3' UTR of mRNAs with poly(A)-binding-protein and modulates ribosomal translation. Under cell stress ATXN2 localizes to stress granules, where the ALS-associated proteins TDP-43 and FUS may also be found.
In order to investigate ATXN2 pathogenesis and identify blood biomarkers of SCA2, we have subjected three patients and three age- and sex-matched healthy individuals from the same family to high-throughput RNA-sequencing. Candidate RNA biomarkers of disease were obtained by unbiased filtering considering only the significance and fold changes, followed by further evaluation on disease relevance. The MMP9 gene, which is a vulnerability factor for motor neurons, appeared to be upregulated by 7.3-fold in SCA2 patients. With a parallel approach, a set of neurodegeneration-associated genes, gathered from Ataxia Exome Panel and ALSoD, was isolated from the transcriptome data; 11 ALS genes showed significant dysregulation in SCA2 patient blood. Validations of these genes with qRT-PCR are being conducted.
There is increasing evidence of ATXN2 involvement in ALS exhibited by its modifier effects on disease duration and on TDP-43, FUS and Profilin1 toxicity, probably through interaction at stress granules. Furthermore, intermediate ATXN2 expansions are enriched in C9orf72 expansion carriers. Making use of the SCA2 patient blood as a model for ATXN2 pathology, we are trying to elucidate the downstream effects of the mutant protein on ALS disease genes and in parallel establish diagnostic tests for clinical routine.

P.46: A NOVEL SPG4 GENE MUTATION CAUSING A UPPER MOTOR NEURON PHENOTYPE WITH NEURONOPATHY. AN ALS MIMIC NOT TO BE MISSED *Verges E, Paipa A, Turon J, Lopez-Toledano E, Povedano M.*

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Case report

A 74 years old man was seen in our clinic because of suspected motor neuron disease. His initial complain was weakness and cramps on both legs. On examination signs of upper motor neuron were found, with paresis on both legs, mainly affecting psoas, and global hyperreflexia. Previous study included MRI of the brain and spinal cord, which was normal, and EMG which was compatible with axonal sensorimotor polineuropathy. Spontaneous activity was also found. Because of these findings a study for spastic paraplegia was started. MLPA was negative for SPG3 , 4, 7. However, on sequentiation a c.284 C>T homocigotic mutation was found. Prompting further study on relatives including two siblings, a man and a woman, both of them asymptomatic and with normal clinical examination. EMG has significant in all of them because of the finding of an axonal sensorimotor polineuropathy. On genetic study the same mutation was found.

Conclusion: We describe a novel SPG4 mutation with dominant inheritance mimicking upper motor neuron disease.

P.47: ALS PHENOTYPE ACCORDING TO HFE P.HIS63ASP POLYMORPHISM: AN ITALIAN MULTICENTRE STUDY *Umberto Manera, Andrea Calvo, Cristina Moglia, Giuseppe Fuda, Mario Sabatelli, Marcella Zollino, ITALSGEN, Silvana Penco, Christian Lunetta, Gabriele Mora, Stefania Battistini, Jessica Mandrioli, Gabriella Restagno, Maura Brunetti, Marco Barberis, Adriano Chio'*

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Objective: To assess the influence of HFE p.His63Asp polymorphism on the phenotype of a large series of Italian ALS patients.
Background: According to a recent study, HFE gene p.His63Asp polymorphism accelerates disease progression in ALS animal models (Nandar et al, Biochim Biophys Acta 2014). Such results were confirmed in a small sample of ALS patients (Su et al, Muscle Nerve 2014).
Design/Methods: We analyzed HFE gene p.His63Asp polymorphism in a sample of 1351 Italian ALS patients, 232 of whom had Sardinian ancestors, to asses genotype differences (CC, GC and GG). Patients with other gene polymorphisms were excluded from the analysis. C9ORF72, TARDBP, SOD1 and FUS mutations were also assessed in all patients. Survival and clinical phenotype were then compared to the patient's HFE polymorphism.
Results: Of the 1351 ALS patients, 363 (29.2%) resulted heterozygous (GC) for the p.His63Asp polymorphism and 30 (2.2%) were homozygous for the minor allele (GG). The number of mutation in the major ALS-related genes were: TARDBP, 75; C9ORF72, 72; SOD1, 26, FUS 14. Subdividing patients according to HFE polymorphism, we found no significant differences for age at onset (61.9 [SD 11.4] vs 62.0 [SD 11.9] years, p= n.s.) and site of onset (bulbar onset, 25.8% vs. 25.7%, p=n.s.). Survival from symptom onset was also similar (p.His63Asp carriers, median survival time 3.4 years [IQR 2.1-6.7]; non-carriers, 3.2 years [IQR 1.9-6.1]). Our results did not change when we considered patients without major gene mutations separately and when we analysed patients from continental Italy and Sardinian patients as distinct subgroups.
Conclusions: In contrast with the results collected in mouse models, we found no effect of the HFE gene p.His63Asp polymorphism on phenotype in ALS patients.

P.48: THE GERMAN PRE-SYMPTOMATIC ALS STUDY (GPS-ALS) 2015 *Patrick Weydt, Melanie Madinger, Christiane Schaldecker, Antje Knehr, Sarah B?hm, Martin Gorges, Jan Kassubek, Jochen Weishaupt, Department of Neurology, Ulm University, Germany, Johannes Prudlo, Department of Neurology, Rostock University, Germany, Peter Andersen, Department of Pharmacology and Clinical Neurosciences, Umea University, Sweden, Albert Ludolph, Department of Neurology, Ulm University, Germany*

Recent advances in the identification of genetic mutations causing autosomal dominant forms of familial amyotrophic lateral sclerosis has opened new opportunities for investigating the presymtomatic phase of the disease. To take full advantage of this novel situation, we are building a national research program for studying these risk carriers. A special challenge arises form aligning the participants right to not know their gene status with the necessity of determining the genotype for further analysis of the data. We have implemented a study design that allows recruitment of people at risk without revealing their gene status and keeping the front-line clinicians also blinded.
For this study ALS-gene risk carriers were recruited from first-degree relatives of ALS patients with known gene mutations (C9ORF72, SOD1, FUS, TDP-43 and others). Regardless of the actual gene status the risk carriers are offered a panel of annual exams consisting of a clinical exam, tissue collection (blood, CSF and others), imaging studies, metabolic test and cognitive exams. The participants and the examiners are blinded to the gene status. Non-mutation carriers serve as internal controls. Genetic counselling is offered to interested parties. A structured phone interview 1 week after the enrolment is used to assess psychosocial effects of the study participation.

To date 40 participants have been enrolled. As expected C9ORF72 and SOD1 are the most common ALS associated mutations in our cohort. Acceptance of the individual tests was high (□90%), except lumbar puncture (□50%). The data and biosamples are being made available to researchers looking for wet and dry biomarkers.
This ongoing study offers the opportunity to investigate the early, presymptomatic (prodromal) phase of ALS while at the same time empowering the growing community of people aware of their risk of developing ALS to participate in the quest for a cure.

P.49: GENETIC MONITORING OF FTLD-ALS SPANIARD PATIENTS *Borrego-Hernandez D.1, Juarez-Rufino, A.1, Atencia, G.1, Cordero-VÁzquez, P.1, Martín, M.A.2, Muñoz-Blanco, J.L.3, Galan, L.4, Esteban-Pérez, J.1 and Garcia-Redondo, A.1*

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Hospital 12 de Octubre ALS Unit (Madrid-Spain) has received samples of ALS patients for genetic testing from 1999.
At present, 129 samples of MND patients associated with FTLD were collected, or ALS or FTD pure patients with any familial case related in first or second degree and linked with the other pathology.
32.56% of total cases correspond to pure FTLD, 28.7% are ALS-FTLD patients, and the rest, 17.05%, are ALS cases.
A number of the samples received have a diagnosis of MND-FTLD. These samples correspond to 17.05 % (3.1% PBP-FTLD, 2.3% PMA-FTLD, 2.3% of PMA and 0.77% of PBP).
We have study SOD1, C9orf72, FUS-TLS, TARDBP, GRN and VCP genes, by Sanger sequencing.
Regarding to genetic analysis, most of positive patients in our genetic study have C9orf72 expansion (10.85%), and only 0.77% has mutations in GRN and SOD1 genes (p.G515A and p.N139H respectively), both in heterozygosity.

P.50: MOLECULAR CHARACTERIZATION OF TUBA4A GENE IN A POPULATION OF ITALIAN PATIENTS AFFECTED BY AMYOTROPHIC LATERAL SCLEROSIS *L. Mosca (1,2), C. Tarlarini (1,2), V. Sansone (2), C2. Lunetta (2), S. Penco (1)*

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Background: Amyotrophic Lateral Sclerosis (ALS) is familial in approximately 10-15% of cases (FALS), while the majority is sporadic (SALS). To date, more than 20 causative genes have been described; C9ORF72, SOD1, TARDBP and FUS are the most commonly mutated in Caucasian populations.

Recently, TUBA4A gene, encoding for tubulin alpha-4A chain, has been described as a novel causative ALS gene; in particular, seven variants were found in 635 FALS patients (1.1%) originating from USA, Canada, Australia and Europe (including Italy).
Purpose: Molecular characterization of TUBA4A gene in a subgroup of Italian ALS patients (FALS and SALS) resulted negative to the screening for the main ALS causative genes (C9ORF72, SOD1, TARDBP, FUS).
Methods: The studied population consisted of 15 FALS and 118 SALS patients; among this last group, 18 cases presented a familial history for neurological diseases including dementia, Parkinson disease and multiple sclerosis.
Four exons of TUBA4A gene have been amplified by PCR and analyzed by direct sequencing.
Results: No pathological mutations have been yet found in the analyzed cases. We identified 9 different genetic variants: 3 were synonymous and 6 were located in non-coding regions of the gene; all variants were reported in public genetic databases.
Conclusions: Our results seem to confirm that mutations in TUBA4A gene are a rare cause of ALS. We propose to analyze the gene in an increasing number of ALS subjects in order to add new frequency mutation data in the Italian ALS population.

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P.51: WHOLE-BLOOD GLOBAL DNA METHYLATION; SELECTION OF ALS PATIENTS FOR GENOME-WIDE METHYLATION PROFILING

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Recent technological advances are allowing researchers to investigate the methylation status of the CpG islands (5'-CpG-3') of the human genome at single nucleotide resolution. Such tools can help identify disease specific epigenetic changes in line with efforts towards solving the mechanisms that govern different complex diseases including Amyotrophic Lateral Sclerosis (ALS). DNA methylation especially at the promoter regions of genes is a well-characterised epigenetic mechanism for gene silencing and is carried out by DNA methyltransferases (Dnmt). These enzymes transfer a methyl group to cytosines located mainly at the CpG islands of gene promoters. On the other hand, another group of enzymes namely the ten-eleven translocation methylcytosine dioxygenase 1 (TET1) are responsible for converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmAC) which is currently under intensive study; aiming to understand its role in epigenetic mechanisms. Keeping in mind the startling heterogeneity of ALS, whether it be holistic or single gene studies, a crucial step in reaching meaningful results is to choose the right set of samples. A good starting point in such epigenetic studies would be to measure the global levels of DNA methylation in specific cohorts of patients; looking for suitable candidates which show aberrant DNA methylation levels. Previous studies on whole-blood global levels of DNA methylation in ALS patients have reported inconsistent results. Here we present data on the performance of different colorimetric and fluorometric DNA ELISA kits designed to detect 5mC and 5hmC levels. Furthermore we report whole-blood global levels of DNA methylation and DNA hydroxymethylation in a large cohort of ALS patients (n=288; both fALS and sALS) and healthy controls (n=96) from Turkey. This study will act as the first step in the further analysis of methylation and the correlating expression profiles in selected ALS patients in a genome-wide manner and at high resolutions via microarray-based technologies.

P.52: THE SPECTRUM OF CLINICAL OPINION ON GENETIC TESTING IN ALS *Alice Vajda, Russell L McLaughlin, Owen Thorpe, Ammar Al-Chalabi, Sharon Abrahams, Orla Hardiman, Trinity College Dublin*

Background: As genetic technologies improve, the number of genes implicated in ALS increases and it is clear that sporadic ALS (SALS) has a strong heritable component, mediated in part by genes known to cause familial ALS (FALS). Nevertheless, genetic testing is often only offered to patients with a clear family history of the disease and variable penetrance, along with unknown pathogenicity for some genes, casts doubt on the utility of genetic testing in some cases.
Aims: To investigate the opinions of ALS specialists towards genetic testing, we surveyed 167 neurologists and other specialists regarding their definition of FALS and genetic testing in FALS, SALS and presymptomatic family members.
Results: The majority of those surveyed (73.3%) did not consider that there is uniform definition of FALS. 57.5% consider a family history of FTD in their definition of FALS. The majority of respondents (90.2%) offer diagnostic testing for patients meeting their definition of FALS, compared to 49.4% who offer it to those with SALS. A substantial proportion (48.5%) of respondents consider testing positive for a known ALS gene to be sufficient to meet the criteria for FALS. Although there was some regional variation in the gene panel tested by respondents, there was a general consensus for the prioritization of four main ALS genes: SOD1, C9orf72, TARDBP and FUS, with relatively few respondents claiming that they test for the other genes included in the survey.
There was significant regional variation in whether respondents would offer presymptomatic testing to ALS families (p = 1.27 , 10-10), and whether they would seek testing themselves were they to belong to an ALS kindred (p = 2 , 10-10).
Conclusions: There is an urgent need for consensus in the definition of Familial ALS, and for guidelines for genetic testing .

PATHOGENESIS

P.53: SOD1 MISFOLDING – A POTENTIAL TARGET FOR THERAPEUTIC INTERVENTION IN ALS *Engel Stanislav, Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

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.
We have developed an innovative computational approach of exploring the dynamic properties of protein surfaces using a steered molecular dynamics simulation (SMD). We demonstrated, in a number of model proteins, that a distinct pattern in which static

residues form defined cluster(s), the so called “stability patches”, surrounded by areas of moderate to high mobility is characteristic of functionally important surface regions involved in protein-protein interaction (PPI) and in binding of small-molecule compounds. In the present work we apply SMD analysis to the field of protein misfolding with the goal of acquiring new insights into the mechanism of noxious gain-of-interaction. We demonstrated that upon misfolding, certain areas of the SOD1 surface acquire characteristic properties of the energetic hot-spots, providing a potential explanation for the gain-of-interaction of misfolded SOD1. Identifying SOD1 surface(s) involved in aberrant PPI may facilitate targeted design of small-molecule inhibitors interfering with the formation of pathogenic protein-protein complexes. 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.54: PRIMARY MOTOR NEURONS EXPRESSING P525L-FUS DISPLAY FUS MISLOCALISATION AND REDUCED SURVIVAL *Louisa Kent, Thomas Vizard, Kevin Talbot, Matthew Thomas, Javier Alegre Abarrategui, Richard Wade-Martins, Kevin Talbot*

Affiliation: Oxford University

Aims: To develop a model of ALS using primary motor neurons with low levels of mutant FUS expression derived from a FUS BAC transgenic mouse model, and to investigate the cellular phenotype of these cells.
Methods: A FUS BAC transgenic mouse model was used to create primary motor neuron cultures expressing wild-type or P525L mutant FUS with an mCherry tag. Cells were stressed with sodium arsenite. Antibodies to mCherry, FET proteins and stress granule markers followed by confocal microscopy were used to assess the distribution of these proteins.
Results: mCherry-tagged wild-type FUS was nuclear in distribution, but P525L-FUS showed marked mislocalisation to the cytoplasm, with only 30% remaining in the nucleus. There is no evidence of mislocalisation of endogenous FUS or other FET proteins. Sodium arsenite treatment led to cytoplasmic granules of P525L-FUS which co-localised with stress granule markers. There is evidence of a reduction in the number of stress granules per cell in the presence of P525L-FUS. Motor neurons expressing P525L-FUS show reduced survival compared to non-transgenic.
Conclusions: These results demonstrate that mutant FUS, even when expressed at very low levels, may become mislocalised in motor neurons and cause defects in stress granule formation and cell survival.

P.55: HUMAN IPS CELLS ALLEVIATE ALS PROGRESSION AND IMPROVE THE STRUCTURE OF AFFECTED EXTRACELLULAR MATRIX *Serhiy Forostyak 1,2, Jessica Kwok 3, Pavla Jendelova 1,2, Ales Homola 1,2, James Fawcett 3 and Eva Sykova 1,2*

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Aims: a) To evaluate the role of the perineuronal nets (PNNs) during the typical course of ALS and after the application of human induced pluripotent stem cells (iPS); b) To study the effects of iPS-transplantation.
Rats' motor function was tested behaviorally throughout the entire course of the disease. Immunohistochemistry was used to visualize spinal PNNs, chondroitin sulphate proteoglycans (CSPGs) and to study the fate of the graft. CSPGs (spinal cord and liquor) were analyzed at the presymptomatic, symptomatic and terminal stages of the disease and in wild-type (WT) littermates. The mRNA expression of CSPGs, growth factors and apoptosis-related genes were analyzed using RT-qPCR. We analyzed the CSF of patients with confirmed diagnosis of ALS and non-ALS controls.
The spinal cord of SOD1 rats showed a different expression of several CSPGs, apoptosis- and growth factor-related genes. The application of iPS cells resulted in significantly better survival, higher motor activity and normalized growth factors' and apoptosis-related genes' expression compared to sham-treated littermates. We found that CSPGs could be detected in the CSF of healthy and SOD1-rats, however at the symptomatic stage transgenic rats had a significantly higher amount of spinal and CSF CSPGs compared to the presymptomatic or age-related WT animals. At the terminal stage rats lost considerably more CSPGs in the spinal cord. Meanwhile, iPS-treated rats had CSPGs levels similar to WT littermates. Our in vivo results were also tested in the human CSF (ALS and non-ALS) where we found differences in some CSPGs expression.
We conclude that the ECM is involved in the pathogenesis of ALS in SOD1 rat model. The administration of human iPS safeguards PNNs and remodels the recipients' pattern of gene expression. Human CSF contains CSPGs and some of them could serve as a biological marker of ALS.

P.56: TDP-43 DISRUPTS ENDOPLASMIC RETICULUM-MITOCHONDRIA ASSOCIATIONS AND CALCIUM HOMEOSTASIS *Radu Stoica, Sebastien Paillusson, Chris Miller Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London*

Mitochondria and the endoplasmic reticulum (ER) form tight structural associations with approximately 5-20% of the mitochondrial surface in close proximity to ER. Molecular scaffolds connecting the organelles can be visualised using electron microscopy and we have recently identified two interacting proteins that tether mitochondria to ER (1, 2). ER-mitochondria associations regulate physiological processes such as calcium and lipid exchange between the two organelles, mitochondrial biogenesis, autophagosome formation, ER stress responses and apoptosis. Interestingly, many of these functions are disrupted in neurodegenerative diseases and attention has recently focused on the ER-mitochondria axis in these disorders. TDP-43 accumulations are a common pathological feature in ALS and frontotemporal dementia (FTD), and mutations in the TDP-43 gene cause some forms of ALS. However, the mechanisms by which TDP-43 induces disease are not clear. We therefore investigated the effects of TDP-43 on ER-mitochondria associations using electron microscopy, immunoprecipitation assays and fluorescence time-lapse microscopy.
We demonstrate that TDP-43 loosens ER-mitochondria contacts and that this is associated with a reduced interaction between proteins tethering mitochondria to ER membranes (2). In addition, the resulting decrease in ER-mitochondria communication leads to altered

intracellular calcium homeostasis. We also show that glycogen synthase kinase-3β (GSK-3β) regulates the ER-mitochondria associations and that overexpression of TDP-43 promotes GSK-3β activity (2).
Thus, we identify ER-mitochondria associations as a new target for damage by TDP-43. Since many physiological processes perturbed by TDP-43 are regulated at the ER-mitochondria interface, the manipulation of ER-mitochondria contacts could provide a new therapeutic approach for ALS/FTD.
References:
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P.57: THE ROLE OF NEUROTROPHIC FACTORS IN EXTRAOCULAR MUSCLE SPARRING IN AMYOTROPHIC LATERAL SCLEROSIS

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In amyotrophic lateral sclerosis (ALS) mouse model, we have recently observed that two neurotrophic factors (NTFs): glial cell line-derived neurotrophic factor (GDNF) and neurotrophin-3 (NT-3) were significantly up-regulated in extraocular muscles (EOMs) of early ALS mice (50 days). The early up-regulations of the two NTFs in EOMs were believed to be associated with the muscles' sparring in late stage ALS mice. Thus NTF was suggested to play an important role in protecting muscle from denervation/degeneration in ALS disease. In the present study, we further examined four different NTFs' (brain derived neurotrophic factor (BDNF), GDNF, NT-3 and neurotrophin-4 (NT-4)) cellular distributions in limb muscle and EOMs from control and ALS mice at 50 days and 150 days. In addition, positive labelling on neuromuscular junctions (NMJs) were quantified and compared between the two muscle tissues from different ages of control and ALS transgenic mice.
The four NTFs were observed in a variety of subcellular structures including NMJs, nerve axons, myotendinous junctions, myonuclei and muscle fibres with different staining intensities. In control mice, proportions of positively labelled NMJs were not significantly changed from early to late stage in either limb muscles or EOMs. In contrast, in ALS mice, all the four NTFs were significantly down-regulated on NMJs of limb muscles from early to late stage. In EOMs of ALS mice, only GDNF was significantly down-regulated on NMJs from early to late stage. Comparisons between control and transgenic mice revealed that expression of BDNF, NT-3, and NT-4 on NMJs were significantly down-regulated in limb muscles but not in EOMs of late ALS mice. The results suggested that NTFs play an important role in reservation of eye muscles anatomy and functions in ALS mice.

P.58: PHOSPHORYLATION OF FUS AFFECTS ITS NUCLEAR IMPORT *Simona Darovic1, Sonja Prpar Mihevc1, Vera Župunski2, Maja Šatlekar1, Youn-Bok Lee3, Christopher Shaw3, Boris Rogelj1*

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Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative disorders with clinical, genetic, and neuropathological overlap. Aberrant cytoplasmic aggregation of FUS (fused in sarcoma) is associated with 3 % of familial ALS and 10 % of all FTLD cases (FTLD-FUS). FUS is a nuclear RNA/DNA binding protein with PY type nuclear localization signal present at its C-terminus which enables interaction with Transportin-1 and its transport into the nucleus. ALS patients with FUS positive cytoplasmic inclusions contain mutations in gene encoding FUS. The majority of these mutations fall within the nuclear localization signal which disables its transport to nucleus. On the other hand, patients with FTLD-FUS do not have FUS mutations but FUS still accumulates in cytoplasmic inclusions, suggesting a different mechanism of inclusion formation in ALS and FTLD. Our aim is to find out whether nuclear localization signal of FUS is subjected to posttranslational modifications that have impact on its localization. We have identified a novel posttranslational modification in nuclear localisation signal of FUS. This modification destroys interaction with Transportin-1 and consequentially affects transport of FUS into the nucleus. Our study implicates new posttranslational modifications as one of the mechanisms by which nuclear transport of FUS is regulated and potentially perturbed in ALS and FTLD.

P.59: NEW INSIGHTS ON HEMATOPOIETIC STEM CELLS DIFFERENTIATION IN TRANSGENIC SOD1G93A MICE *Gasco S1, Calvo AC1, Rando A1, Oliván S1, Toivonen JM1, Esteban Pérez J2, Zaragoza P1, García Redondo A2, Osta R1*

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Blood is the latest explored tissue in the prognosis and diagnosis of ALS and therefore it can favour an accurate translational study to human samples. Hematopoietic stem cells (HSC) are multipotent cells with self-renewal capacity that can give rise to their closest oligopotent hematopoietic progenitor cells, best known as Common Lymphoid Progenitors (CLP) and Common Myeloid Progenitors (CMP). In this study we propose to investigate the frequencies of HSC, CLP and CMP in a murine model along disease progression. The main aim of this study was to characterize for the first time the frequency of HSC, CLP and CMP in

serial blood extractions from transgenic SOD1G93A mice. Blood serial samples from transgenic mice were collected first at the age of 30 days to study the frequency of HSC, CLP and CMP by real time PCR and flow cytometry along disease progression. Two-tailed t-Student tests were used to assess statistical significance between groups. A total of 40 mice were included in this study, 20 control and 20 transgenic mice of both sexes. The results obtained in serial blood samples suggested at transcriptional level, HSC were almost significantly activated in transgenic mice at terminal stage ($p=0,06$). Interestingly, in the flow cytometry study, the frequency of HSC was significantly increased since symptomatic stage to the end-point of transgenic mice ($p<0,01$). However, the frequencies of CLP and CMP decreased along disease progression, starting at late ($p<0,05$) and early symptomatic stage ($p<0,05$), respectively. The findings could suggest a time-point dependent differentiation of HSC to CLP and CMP, which could be influenced by the degenerative progression of the disease. This time dependent activation of HSC could shed light on the role of hematopoietic system in the progression of ALS and future therapeutic targets could be defined.

P.60: DIRECT INTERACTION BETWEEN MACROPHAGE MIGRATION INHIBITORY FACTOR AND SOD1 *Guy Zoltsman and Adrian Israelson Department of Physiology and Cell Biology, Ben-Gurion University of the Negev, Israel*

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 the misfolded SOD1 association to the mitochondria and ER membranes. Now, we used microscale thermophoresis (MST) and surface plasmon resonance (SPR) assays in order to characterize the interaction between SOD1 (wild type, SOD1G93A and SOD1G85R) and MIF in vitro. Our next goal is to identify the binding region between MIF and SOD1. We believe this information will lead to a better understanding of the misfolded SOD1 toxicity mechanism, and will contribute for the future development of MIF-based therapies for ALS.

P.61: SMAD PROTEINS MEDIATE TGFBETA1 EFFECTS IN ALS MUSCLE *Meroni M., Cicardi M., Cristofani R., Crippa V., Rusmini P., Messi E., Poletti A., Galbiati M. Dipartimento di Scienze Farmacologiche e Biomolecolari - Centre of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, Italia. Inter-University Research Centre on the Molecular Basis of Neurodegenerative diseases (Universities of Florence, Rome and Milan, Italy)*

Some familiar forms of Amyotrophic Lateral Sclerosis (ALS) are characterized by a dominant mutation in superoxide dismutase (SOD) 1 gene. Many evidence suggested that SOD1 toxicity is non-cell autonomous, involving multiple cell types: motoneurons, glial cells and muscle cells. In particular, muscle might be a primary source of toxicity, since it is reported that in mice the expression of mutated SOD1 in muscle cells is sufficient to induce motorneuron degeneration and muscle abnormalities already at pre-symptomatic stages.

TGFbeta1 is a growth factor known to be involved in neuron survival and in muscle development/maintenance. Furthermore, TGFbeta1 levels are increased in ALS patient serum. Therefore we decided to evaluate the expression of TGFbeta1and Smads (which are involved in the main signal transduction pathway) in the skeletal muscles of transgenic mice expressing mutated hSOD1. The results indicate that mutated hSOD1 up-regulates the expression of TGFbeta1already at the pre-symptomatic stage. The expression of mutated hSOD1 correlated with a decrease of Smad 2 and 4, while the Smad 3 mRNA levels increase. Our previous results indicate that proteasomial and autophagic activity are higher in muscle cells than motorneuron. For this reason we decided to evaluate the effect of TGFbeta1 on the protein quality (PQC) systems of the C2C12 myoblasts measuring the gene expression of different markers. The data indicate that TGFbeta1 is able to modulate the expression of p62, HSPB8, and atrogin, without affecting BAG3 and BAG1 mRNA levels.
Our results suggest that ALS skeletal muscles have an increased expression of TGFbeta1, but the action of this growth factor could be impaired by the low level of Smad4 that is necessary to import the TGFbeta1 into the nucleus. Moreover we have demonstrated that TGFbeta1 is able to modulate the PQC pathways.
Grants: ARISLA, Telethon-Italy, Fondazione Cariplo, AFM.

P.62: BID POSITIVELY REGULATES THE TLR – NF-KAPPAB INFLAMMATORY RESPONSE IN A SOD1G93A ALS MODEL *Sinéad Kinsella, Hans-Georg Koenig, Jochen H. M. Prehn. Dept. of Physiology and Medical Physics, RCSI.*

There is evidence of increased microgliosis and astrogliosis in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). Elevated levels of Toll-like Receptors (TLRs) -2 and -4, master regulators of immune response, were identified in the brain and spinal cord in ALS pathology. The Bcl2 family member Bid, previously implicated in death receptor – induced apoptosis, has recently been shown to induce inflammation via positive regulation of the IKK component of the TLR-NF-kappaB pathway. This study examined TLR-induced and Bid-dependent microglial activation in response to ALS-mutant Superoxide Dismutase 1 (SOD1G93A) in vitro. We observed increased tlr2 and tlr4 expression in response to SOD1G93A overexpression in microglial cells (BV2). Interestingly, expression of the NF-kappaB target gene, COX-II, is decreased in BV2 cells following Bid siRNA transfection and subsequent stimulation with motoneuron SOD1G93A-conditioned media compared with control siRNA-transfected cells exposed to the same stimulus. Our data shows a delayed degradation of IkappaBalpha and a significant reduction in the phosphorylation of IKKalpha/beta and p65 in bid-/- microglia compared to wt microglia upon acute LPS stimulation. Additionally, we report a significant reduction in the levels of Peli1, which is involved in the NF-kappaB activating polyubiquitination of the IRAK complex, in bid-/- microglia compared with wt upon both acute and prolonged LPS stimulation. Furthermore, our data provides evidence for protein interaction between Bid and the E3 ubiquitin ligase TRAF6, with Bid promoting TRAF6 polyubiquitination. These data demonstrate that TLR signalling in microglia is activated by overexpressed SOD1G93A, and reveal that Bid positively regulates the TLR–NF-kappaB signalling in microglia.

P.63: ALTERED CHLORIDE HOMEOSTASIS IN EMBRYONIC SPINAL SOD1G93A MOTONEURONS *Elodie Martin, William Cazenave, Anne-Emilie ALLAIN, Daniel Cattaert, Pascal Branchereau Univ. Bordeaux, INCIA, UMR 5287, F-33615 Pessac, France. CNRS, INCIA, UMR 5287, F-33615 Pessac, France*

Amyotrophic lateral sclerosis (ALS) is an incurable paralytic disease caused by degeneration of motor neurons that usually exhibits clinical symptoms during adulthood. As a consequence, most studies have focused on peri-symptomatic periods, revealing numerous abnormal cellular mechanisms. But an early initiation of pathology in motor neurons accompanied by compensatory mechanisms can be the scenario of the ALS disease. Based on this hypothesis, we analyzed the maturation of embryonic motor neural networks in SOD1G93A mouse, a reliable model of ALS disease, at prenatal stage when locomotor networks become mature and when spinal motoneurons (MNs) acquire their adult features. We have previously shown, in mice, that during this ontogenic period, the GABA/glycine synaptic transmission underlies a profound change leading to the appearance of inhibition (Delpy et al., J. Physiol. 2008). The two main chloride co-transporters (KCC2 that extrudes chloride from neurons and NKCC1 that uptakes chloride) were involved in this change. Our results indicate that, when compared to WT from the same littermate, SOD1G93A mouse exhibit altered chloride equilibrium potential (ECl) accompanied by a modification of chloride co-transporters, both at the level of the protein expression and efficacy. Although left/right alternated locomotor activity likely relies on inhibition between ipsi- and contra-lateral half centers, our preliminary data point out that locomotor activity is present in SOD1G93A spinal cords maintained in vitro. Altogether, our results indicate that chloride homeostasis is affected early in development in SOD1G93A MNs while locomotor networks keep functional, suggesting that deep compensatory mechanisms occur in ALS mouse model when motor networks emerge.
The microscopy was done in the Bordeaux Imaging Center. The help of Christel Poujol and Sébastien Marais is acknowledged.
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P.64: UPREGULATION OF MICRORNA-155 PRECEDES SYMPTOMATIC INFLAMMATION IN THE SPINAL CORD OF TGSOD1G93A MICE *Carolina Cunha1, Catarina Santos1, Adelaide Fernandes1,2, Cátia Gomes1, Filipe Nascimento3,4, Ana M. Sebastião3,4, Ana Rita Vaz1,2, Dora Brites1,2*

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Neuroinflammation is a pathological hallmark of amyotrophic lateral sclerosis (ALS) either familial (fALS) or sporadic (sALS). Recent studies identified microRNA (miR)-155 as critical in

neuroinflammation and a key feature in TgSOD1G93A (mSOD1) mice (at end-stage), as well as in sALS and fALS patients.
Here we aimed: (i) to explore miR-155 as an early biomarker of ALS; (ii) and to investigate miR-155 associated neuroinflammatory pathways. Spinal cord samples were collected from the mSOD1 mice at pre-symptomatic (4-5 weeks) and symptomatic (14-15 weeks) stages and analyzed by WB and qRT-PCR.
Our results indicated that the three major inflammatory miR-155, miR-124 and miR-146a significantly increase in symptomatic mSOD1 mice, but only miR-155 was upregulated in pre-symptomatic phase, together with the downregulation of its direct target SOCS1, in both stages. Interestingly, while suppression of TGF-β in pre-symptomatic stage may derive from miR-155 signalling, its upregulation in the symptomatic one can in opposite induce miR-155 expression. Moreover, miR-155 overexpression in the pre-symptomatic stage may cause a decreased clearance by microglia as it is suggested by the reduced expression of MFG-E8, essential in the phagocytosis of apoptotic cells. Most importantly, these findings were shown to precede the polarization of microglia towards the M1 pro-inflammatory phenotype that we observed in the symptomatic stage, manifested by the increased expression of CD11b, Iba1, MHC-II and C/EBPα, together with the decrease in arginase-1 expression. Enhanced expression of the CX3CL1-CX3CR1 axis, HMGB1 alarmin, inflammasome-NLRP3 and IL-1β corroborated the inflammatory milieu at the symptomatic, but not at the pre-symptomatic stage.
Data suggest miR-155 as a biomarker for early diagnosis of ALS and highlight its potential as a therapeutic target. Further studies are needed to demonstrate the clinical value of the circulating miR-155 in ALS patients.
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P.65: SEARCH FOR MIRNA-REGULATED MOLECULAR NETWORKS IN ALS AND PRION DISEASE *Toivonen JM1, Calvo AC (1), Oliván S1, Manzano R (1), Sanz-Rubio D (1), Espejo-Porras F (2), De Lago E (2), Martín-Burriel I (1), Zaragoza P (1), Osta R (1).*

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Prion diseases, or transmissible spongiform encephalopathies (TSE), are caused by misfolding of a native prion protein PrPC to a pathological, infectious conformation. Several proteins affected in human neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), contain prion-like domains. Pathogenic prions can impair retrograde transport in the axons projecting via spinal cord, a feature also observed in ALS. Although the affected CNS regions differ between ALS and prion disease, molecular pathology involved may be partially related. Prion-like proteins associated with ALS, such as TDP-43 (TARDBP) and FUS, have propensity to form aggregates and induce misfolding/aggregation in other proteins (including SOD1) and spread to neighbouring cells using prion-like propagation. We have previously analysed circulating plasma microRNAs (miRNA) in

mSOD1 mice and showed that some miRNA implicated in maintenance of neuromuscular junction and in amyloid beta metabolism could serve as potential biomarkers in ALS mice and human patients. Unlike SOD1, TDP-43, FUS and PrPC closely engage with molecular machinery involved in miRNA production: TDP-43 promotes miRNA biosynthesis and PrPC facilitates the assembly of miRNA effector complexes. From the above it seems feasible that search miRNA biomarkers in ALS and TSE models may mutually advance our understanding of ALS and prion diseases. In the future our aim is to study miRNA regulation in models of ALS and TSE in order to dissect shared and private mechanisms of miRNA-regulated molecular pathways in the CNS and to investigate the applicability of these miRNAs for circulating (plasma or exosomal) biomarkers.

P.66: THE ROLE OF CASPASE 6 IN ALS *Marion C Hogg 1, Mollie R Mitchem 1, Hans-Georg König 1, & Jochen H.M.Prehn 1,2.*

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The precise cause of motoneuron cell death in ALS remains elusive but caspase-dependent pathways have been implicated amongst others. Recent work indicated that caspase 6 plays a role in axonal degeneration. Therefore we hypothesised that caspase 6 may be involved in motoneuron cell death in ALS. To investigate the role of caspase 6 in ALS we profiled protein levels of caspase-6 throughout disease progression in the ALS mouse model SOD1G93A; this showed elevated levels of full length caspase 6 were present at the end stage of disease. Activated, cleaved caspase 6 could not be detected at any stage of the disease. We also found that caspase-6 was particularly enriched in microglia cells rather than astrocytes or motoneurons. To investigate further we used capsase 6 null mice and created a crossbred colony with the SOD1G93A model. Surprisingly, analysis of the transgenic SOD1G93A/c6-/- revealed an exacerbated phenotype with motor dysfunction occurring earlier and a significantly shortened lifespan when compared to transgenic SOD1G93A/c6+/+ mice. Immunofluorescence analysis of the neuromuscular junction revealed no obvious difference between caspase 6 +/+ and -/- in non-transgenic mice, whilst the SOD1G93A transgenic mice showed severe degeneration compared to non-transgenic mice in both genotypes. Our data indicate that caspase-6 is not required for ALS pathogenesis, but may play a protective role during disease progression.

P.67: EVALUATION OF PRION-LIKE TRANSMISSIBILITY IN ALS: INSIGHTS FROM LABEL-FREE PROTEOMIC ANALYSIS *Luciana Pizzatti1, Auderlan Mendonça de Góis2, Thais Andrade de Lima2, Luciana Oliveira2, José Suelton Luiz Costa dos Santos2, José Fabio Santos Leopoldino3, Marli Pernes4, Claudio Heitor Tavares Gress4, José Mauro Braz de Lima4, Regina Helena da Silva5, Alessandra Mussi de Ribeiro5, Eliana Abdelhay6, Leila Chimelli7, José Ronaldo dos Santos2, Deise Maria Furtado de Mendonça2. Departamento de Química, Instituto de Química, Universidade Federal do Rio de Janeiro7; Departamento de Biociências, Universidade Federal de Sergipe2; Hospital Universitário de Sergipe, Universidade Federal de Sergipe3; Instituto de Neurologia Deolindo Couto, Universidade Federal do Rio de Janeiro4; Departamento de Fisiologia, Universidade Federal do Rio Grande do Norte5; Divisão de Laboratórios do CEMO, Instituto Nacional do Câncer6; Instituto Estadual do Cérebro/Rio de Janeiro7.*

The mechanism underling ALS pathogenesis is still unknown. Therefore, it is clear that formation of aberrant protein aggregates is a pathway which links the neurodegeneration process in all clinical forms of the disease. It has been hypothesized that misfolded protein assemblies could act as seeds of aggregation. It is possible that these seeds could sequester their native protein isoforms, convert them into pathological molecules and, after that, they could be released to the extracellular space, acting as prion-like agents. In this way, Cerebrospinal Fluid (CSF) comprises proteins expressed by nervous system cells that could be related to the pathogenic mechanism and spreading of disease. In our study, we intend to evaluate prion-like transmissibility in ALS. We performed intraventricular injections in Wistar rats of CSF from an ALS patient. Sixteen Wistar middle aged rats were assigned to one of 2 groups (n = 8 per group): a control group, injected with artificial CSF, and an ALS group, injected with CSF of an ALS sporadic patient. The animals received one injection i.c.v. of 5µl of vehicle or CSF (healthy controls or ALS patient). After one month, rats were submitted to blood removal. Blood samples were centrifuged and serum from all animals were processed to Label-Free LC-MS strategy for determining the proteomic profile of both groups. About two hundred differentially expressed proteins were identified in ALS group when compared to control group. Proteins like Neurofilament Heavy Subunit (NF-H), Glial Fibrillary Acid Protein (GFAP), Microtubule associated protein 2 (MAP 2), Dynein Heavy Chain 12, Axonemal (Dnah 12), Ubiquitin 60S ribosomal protein L40, and Notch 2 were found. Some of these proteins found in ALS group is known to be involved with ALS. All the proteins found and signaling pathways that are involved in the disease as well as transmissibility in ALS must be investigated.

P.68: EFFECT OF PROTEIN QUALITY CONTROL SYSTEM ON TDP43 ACCUMULATION IN ALS MOTONEURONAL AND MUSCLE MODELS *Maria Elena Cicardi, Valeria Crippa, Rita Galbiati, Marco Meroni, Riccardo Cristofani, Paola Rusmini, Angelo Poletti. Sezione di Biomedicina ed Endocrinologia, Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Centro di Eccellenza sulle Malattie Neurodegenerative, Università degli Studi di Milano, Milano, Italy. Centro InterUniversitario sulle Malattie Neurodegenerative, Università degli Studi di Firenze, Milano, Genova e Roma Tor Vergata, Italy*

Recent studies have shown muscle involvement at onset and progression of ALS. We focused our attention on TDP43 which is mislocalized and aggregates in the cytoplasm. The protein quality control (PQC) system is responsible for the correct protein homeostasis. The

chaperones maintain proteins in the correct folding. If this system fails chaperones drives misfolded proteins to the degradative systems: ubiquitin-proteasome system (UPS) and autophagy. We characterized PQC system behavior in presence of TDP43-full length or TDP43-25, a pathological fragment present in familial ALS cases.
Initially, we compared the activity of the PQC system in motoneurons NSC34 and muscle C2C12 cells. By RTq PCR, Western Blot and Immunofluorescence analyses of key protein involved in the degradative systems (Bag3, Bag1, p62 and LC3) we found that muscle cells have a more activated autophagic system than motorneurons.
Then, we studied the behavior of the PQC system in presence of TDP43 full length or TDP43-25 fragment. We pharmacologically inhibited degradative systems and by Filter Trap Assay and Western Blot analysis we observed that both TDP43 species are primarily degraded trough UPS.
Immunofluorescence analysis showed that UPS inhibition increased TDP43 levels and induced accumulation of the truncated protein in the cytoplasm.
We also investigated chaperones involvement in the removal of toxic proteins. We have already shown that overexpression of the small heat shock protein B8 (sHSPB8) reduces TDP43 aggregation in motoneurons. Surprisingly, we noted that HSPB8 overexpression had no effect on TDP43 aggregation. Silencing HSPB8 in muscle cells produced no effect on the aggregation of both proteins.
In conclusion, we demonstrated that PQC system has a key role in the removal of pathological protein. We also demonstrated that degradative systems could be differentially activated in motoneuronal or muscle cells.
GRANTS: Regione Lombardia; AFM-TELETHON; FONDAZIONE TELETHON; FONDAZIONE CARIPLO; FONDAZIONE ARISLA; Ministero della Sanit.

P.69: GOLGI PATHOLOGY IN SOD1-ALS IS MEDIATED BY STATHMINS 1/2, MICROTUBULES AND GOLGI SNARES *Sarah Bellouze, Gilbert Baillat, Dorothee Buttigieg, Nathalie Cavanne, Catherine Rabouille, Georg Haase Institut de Neurosciences de la Timone, CNRS and Aix-Marseille University, Marseille, France. Hubrecht Institute for Stem Cell Research and Developmental Biology, Utrecht, The Netherlands.*

Pathological Golgi alterations represent an early and constant feature of degenerating motor neurons in amyotrophic lateral sclerosis (ALS) and related disorders. A pathogenic role of microtubules has been suspected since microtubules are closely associated with Golgi membranes and since microtubule-depolymerizing drugs trigger Golgi disruption.
We here demonstrate severe Golgi fragmentation and atrophy in lumbar motor neurons of transgenic SOD1 G85R and G93A ALS mice. In contrast to their wildtype counterpart, the SOD1 mutants localize to Golgi membranes, impede the growth of Golgi-derived microtubules and cause a reduction in polymerized microtubules in motor neuron cultures. This in turn leads to accumulation of the Golgi v-SNARE proteins GS15 and GS28 which are involved in ER-Golgi trafficking. The pathological Golgi alterations are mediated at least in part by the microtubule-destabilizing proteins Stathmin-1 and -2, since both proteins show progressively up-regulation together with GS15 and GS28 in vivo and when overexpressed in vitro cause the same microtubule disruption, Golgi fragmentation and GS accumulation as SOD1 mutants.
This study identifies microtubule defects in SOD1-linked Golgi pathology and points to degenerative mechanisms shared with other motor neuron diseases, i.e. stathmin-1 up-

regulation in spinal muscular atrophy (SMA) and defective tubulin polymerization in progressive motor neuronopathy and TUBA4A-linked ALS.

P.70: THE ROLE OF SIGMA-1 RECEPTOR IN THE ER-MITOCHONDRIA CALCIUM CYCLE IN THE G93A MOUSE MODEL OF ALS *Tadic V (1), Malci A (1), Prell T (1), Liu J (1), Witte OW (1,2), Grosskreutz J (1)*

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Background: Ca2+ disturbances, ER stress and mitochondrial dysfunction play a central role in the pathophysiology of ALS. Cell survival is dependent on bioenergetics that is controlled by fine signaling in ER-mitochondria calcium cycle (ERMCC). The sigma-1 receptor (Sigma-1R) modulates Ca2+ homeostasis affecting mitochondrial Ca2+ influx by stabilizing IP3R. Mutations in SIGMAR1 have also been identified in ALS-FTLD. Pharmacological manipulation of the receptor was neuroprotective in G93AhSOD1 and wobbler mice.
Objective: The aim of our study was to dissect the function of Sigma-1R in ERMCC in the presence and absence of mutated hSOD1 in motor neuron culture.
Material and methods: Mouse spinal motor neurons from E13 mouse embryos (non-transgenic and G93AhSOD1) were seeded on a glial feeder layer. Single cell neuronal cytosolic Ca2+ dynamics were monitored using fura 2-AM. The cells were repetitively stimulated with AMPA receptor agonist kainate and with bradykinin. The same experiment was repeated after incubating the cells with Sigma-1R agonists PRE-084 or SA 4503.
Results: Bradykinin induced Ca2+ response was altered in the presence of mutated hSOD1. Incubation with PRE-084 weakened bradykinin induced Ca2+ response in non-transgenic neurons. PRE-084 incubation did not affect bradykinin induced Ca2+ response in G93AhSOD1 neurons, but it has enlarged kainate induced Ca2+ response in G93AhSOD1 motor neurons. Treatment with PRE-084 did not influence viability of motor and non-motor neurons in non-transgenic and G93AhSOD1 cell culture.
Activation of Sigma-1R with another agonist, SA 4503, rescued bradykinin induced Ca2+ response in SOD1 neurons.
Discussion and conclusion: Sigma-1R contributes in ERMCC and influences neuronal calcium homeostasis. From the results it is evident that two agonists have different effects on ERMCC in cultured motor neurons and that Sigma-1R has different roles in non-transgenic and G93AhSOD1 neurons. The dual role of the Sigma-1R needs to be investigated in details using advanced pharmacological tools.

P.71: SOD1 REDUCTION MORPHOLINO-MEDIATED AMELIORATES AMYOTROPHIC LATERAL SCLEROSIS DISEASE PHENOTYPE *M. Nizzardo, C. Simone, F. Rizzo, Ulzi G., A. Ramirez, M. Bucchia, A. Bordoni, N. Bresolin, G. P. Comi, and S. Corti For all the authors: Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), University of Milan, Neurology Unit, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder caused by motor neuron degeneration. No effective treatments are presently available.

The vast majority of cases are sporadic (SALS), while 10% are familial ALS (FALS) (Renton et al., 2014). Numerous studies identified mutations in different genes, in particular the majority are located in the gene encoding for Cu/Zn superoxide dismutase 1 (SOD1). Mutations in SOD1 gene are typical in FALS and lead to a progressive MN death, due to one or more acquired toxicities.
Recently misfolded SOD1 has been found in affected tissue of sporadic patients, suggesting a concrete pathogenetic role for those SOD1 species (Pokrishevsky et al., 2012). For these reasons, it represents a promising therapeutic target for antisense oligonucleotides.
The strategy we adopted was the administration of Morpholino oligonucleotides in in vivo ALS models, in order to reduce the synthesis of ALS-causing human SOD1. This technique allowed us to demonstrate an increase of survival, with a consequent amelioration of neuromuscular functions and a slowdown of the disease progression. Neuropathological analysis demonstrated an increased motor neuron and axon number, an ameliorated muscle trophism and a reduced micro and macrogliosis-mediated inflammation.
The SOD1 suppression, in particular of misfolded form, caused by the use of Morpholino is visible in vivo in ALS rodent. These results are the first step for Morpholino-mediated therapy in human clinical trials.

P.72: A ROLE FOR ER SHAPING PROTEINS IN MITOCHONDRIAL NETWORK ORGANIZATION

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Hereditary spastic paraplegias (HSP) are a group of neurodegenerative disorders characterized by retrograde degeneration of long motor neurons within the corticospinal tract, leading to muscle weakness and spasticity in the lower limbs. A common cause of HSP includes mutations in endoplasmic reticulum (ER)-shaping proteins that induce ER curvature via an intramembrane hairpin loop domain. However, the mechanism through which loss of these ER-shaping proteins and motor neuron degeneration as seen in HSP remains unknown. ARL6IP1 has recently been identified as an HSP causing gene, with mutations in ARL6IP1 linked to SPG61. We have therefore generated a novel model of HSP by RNAi knockdown of Arl6IP1 in the fruit fly Drosophila melanogaster. In this model flies display progressive locomotor deficits, highlighting their usefulness as a model to study this disease. Using this model and established (Reticulon) models of HSP we also show that a loss of these ER-shaping proteins disrupts ER organization, with altered smooth ER staining at the distal ends of motor neurons. Furthermore, we show that both Arl6IP1 and Reticulon are required for mitochondrial organization within the distal ends of motor neurons. To validate the functional conservation of these proteins, we have also investigated the effect of loss of ER-shaping proteins on ER and mitochondrial organization in a mammalian cell model. Taken together, these findings suggest that ER-shaping proteins are important for mitochondrial network organisation and ER-mitochondrial contacts may be a common mechanism in the pathogenesis of HSP.

DISEASE MODELS

P.73: TRANSCRIPTOME OF MOTOR NEURON SOMAS AND AXONS DERIVED FROM EMBRYONIC STEM CELLS USING MICROFLUIDICS

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Motor neurons are highly polarized cells that initiate body movement through interaction with muscles by specialized synapses termed neuromuscular junctions (NMJs). Anatomically motor neurons can be subdivided into distinct compartments – the cell body (soma) and associated dendrites, the axon and the synaptic terminal. Each compartment of the motor neuron performs distinct functions. Motor axons are provided with efficient biochemical machinery, since they fulfill highly demanding metabolic and physiological events that take place at the presynaptic terminal. However, the axonal RNA composition of motor neurons is still largely unknown. Motor axons and NMJs appear to be primary targets in the motor neuron disease amyotrophic lateral sclerosis (ALS), with muscle endplates becoming denervated before onset of central motor neuron loss and axons showing altered biology. Some of the early axonal deficiencies reported to date involve local RNA processing and transport. In our study, we cultured spinal motor neurons derived from mouse embryonic stem cells (mESCs), in a microfluidic device and performed deep-RNA sequencing (RNAseq) on somatodendritic and axonal compartments separately. We used mESCs overexpressing the human mutated (G93A) superoxidase dismutase 1 (SOD1) gene to model ALS. In parallel to the RNAseq analysis, we characterized the motor neuron cultures using electrophysiology. In summary, we believe that our work will give new insights into motor axon organization of in health and ALS.

P.74: THE SPHINGOSINE 1-PHOSPHATE RECEPTOR 1 (S1P1) AGONIST FTY720 (FINGOLIMOD) IN A RAT MODEL OF ALS

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Background: Interactions of the peripheral immune system with the central nervous system are thought to affect the progression of motoneurodegeneration in amyotrophic lateral sclerosis. S1P1 agonists block the egress of lymphocytes from lymphoid organs and act directly on astrocytes of the brain. A brain penetrating S1P1 agonist may thus influence both T-cell-microglia interactions and astrocytosis at sites of neuroinflammation.
Objective: Evaluate the potential therapeutic effect of fingolimod in a transgenic ALS rat model based on overexpression of mutant human superoxide dismutase 1 (hSOD1G93A).
Methods: Male hemizygous hSOD1G93A rats (n=36) and their wild-type littermates (n=14) were treated with fingolimod at 1 mg/kg/day (food-admix) or vehicle starting from 90 days of age (pre-symptomatic phase) until reaching end-stage of disease (loss of mobility). The study was terminated at 246 days of age when 90% of hSOD1G93A rats had reached the end-stage criterion. The remaining transgenic rats were censored in the survival analysis. The onset and progression of disease were analyzed by regular body weight and grip strength measurements and by general neurological assessments. Fingolimod plasma and brain concentrations, as well as blood lymphocyte counts were evaluated in a subset of transgenic and wild-type rats.

Results: Fingolimod at 1 mg/kg/d effectively reduced lymphocyte counts in peripheral blood of both wild-type and transgenic rats. Fingolimod did not affect disease onset and progression as measured by neurological signs, or survival time, in transgenic rats. Fingolimod slightly decreased the body weight and grip strength of transgenic rats without affecting those measures in wild-type rats.
Conclusion: The S1P1 agonist fingolimod at a dose resulting in significant systemic lymphocyte decrease did not modify disease onset, progression or survival in hSOD1G93A transgenic rats, a pathogenic model of familial ALS.

P.75: DEREGULATED GENE/PROTEIN EXPRESSION IN ASTROCYTES FROM MSOD1 MICE PUPS MIMIC THE SYMPTOMATIC STAGE *Cátia Gomes1, Carolina Cunha1, Filipe Nascimento2,3, Ana Sebastião2,3, Ana Rita Vaz1,4, Dora Brites1,4*

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Increasing evidences suggest that dysfunctional astrocytes are key players in motor neuron (MN) degeneration in Amyotrophic Lateral Sclerosis (ALS). Astrocytes isolated from the spinal cord of SOD1G93A (mSOD1) rodents at symptomatic stage were described as phenotypically aberrant (AbAstro/Tox cells), with higher reactivity and toxicity to MN.
We investigated whether AbAstro/Tox cells were represented in the population of cortical astrocytes isolated from neonatal pups and if so, at what extend reproduced the gene/protein deregulated profile of the mSOD1 mice either at pre-symptomatic or at symptomatic stages.
Astrocytes were isolated from the cortex of 7 day wild-type and mSOD1 mice, and cultured for 13 days. Cortical brain samples were collected from the same animals at pre-symptomatic (4-5 weeks) and symptomatic (14-15 weeks) stages.
Accumulation of SOD1 was observed in the pre-symptomatic stage, but mainly at the symptomatic stage and in astrocyte cultures. These cells evidenced decreased expression of GFAP and GLT-1, as well as higher SOD1 accumulation, as compared with wild-type cells. In addition they revealed increased expression of vimentin, S100B, Cx43, high-mobility group box 1 (HMGB1) and the proliferative marker Ki-67, reinforcing the prevalence of reactive AbAstro/Tox cells. Such gene/protein deregulated profile was similarly observed in the cortical tissue of the mSOD1 mice at the symptomatic stage, but not at the pre-symptomatic one. Down-regulation of the microRNA(miR)-146a, but not of miR-155 or miR-124, was again a common feature in astrocytes and symptomatic cortical tissue.
Our data indicate AbAstro/Tox cells as having a key role in ALS disease progression, and specify miR-146a as a potential target for the development of novel drugs. We anticipate the engineering of AbAstro/Tox cells toward a neuroprotective phenotype as a potential therapeutic strategy in ALS.
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P.76: MODELING MOTOR NEURON DEGENERATION IN AMYOTROPHIC LATERAL SCLEROSIS USING EMBRYONIC STEM CELLS *Ilary Allodi1, Jik Nijssen1, Ming Cao1, Andrea Fuchs2, Ole Kiehn2 and Eva Hedlund1*

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Pluripotent stem cell-derived neurons can be used to model diseases, elucidate mechanisms of neurodegeneration and perform drug screens in vitro. In Amyotrophic Lateral Sclerosis (ALS) a majority of motor neurons (MNs) in the spinal cord and brainstem, that innervate voluntary muscles, degenerates, while oculomotor neurons (OM MNs) in the midbrain are spared. The reasons for this differential vulnerability to disease are unknown. The goal of our studies was to derive MN populations with different vulnerabilities to degeneration in ALS from mouse embryonic stem cells (mESCs) and model their differential susceptibility to disease in vitro. Using extrinsic and intrinsic signals known to specify distinct MN populations along the anterior-posterior axis of the embryo, we could direct differentiation of our mESC cultures. Specifically, spinal MNs were patterned using retinoic acid (RA) and sonic hedgehog (Shh) and OM MNs using Shh and fibroblast growth factor 8. Forced expression of the intrinsic determinant Phox2A increased the yield of resistant OM MNs, while Olig2 further directed mESCs into spinal MNs. The identities of the MNs were confirmed by staining for subpopulation specific markers and by electrophysiological recordings. Furthermore, we are developing in vitro assays in which these MN populations should show differential vulnerability to ALS-like toxicity. We believe that these in vitro systems will aid in identifying future targets for the treatment of ALS, as they allow gain- and loss-of-function studies for candidate genes that could play a role in MN protection and vulnerability.

P.77: PGC-1ALPHA EXPRESSION AND FUNCTION IN MOUSE MODELS OF AMYOTROPHIC LATERAL SCLEROSIS *Hanna Bayer(1), Kerstin Lang(1), Johannes Hanselmann(1), Irma Merdian(1), Luc Dupuis(2), Patrick Weydt(1), Anke Witting(1)*

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Genetic variants of a brain-specific promoter of peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-α (PGC-1α) modify age of onset in human ALS(1) suggesting that a changed function or activity of PGC-1α contributes to the pathogenesis of ALS. We investigated the PGC-1α expression and function in different tissues and cells of two ALS mouse models, SOD1(G93A) mice and a knock-in Fus mouse model deleted of the nuclear localization signal (FusΔNLS).
We showed that the canonical and brain-specific PGC-1α isoforms are reduced in spinal cord and brain stem of diseased mice from the SOD1 mouse model, whereas PGC-1α remains unchanged in unaffected brain regions and in non-diseased FusΔNLS/+ mice. In primary neurons and glia cells of these mouse models the basal expression of PGC-1α was unchanged in comparison to corresponding controls. To investigate the effect of ALS associated mutations on a stimulated PGC-1α signaling we identified lactate as a neuron-specific stimulus. The lactate induced PGC-1α expression and signaling was not influenced

by the tested ALS associated mutations. In contrast to brain, PGC-1α was increased in brown fat and muscle of diseased SOD1 animals. Investigations on the effect of ALS associated mutations on the basal or stimulated PGC-1α signaling in primary myoblasts and brown adipocytes are currently under investigation.
So far, our results suggest that changes in PGC-1α during the course of disease are not primarily due to ALS associated mutations in SOD1 and Fus.
References: 1. Eschbach J, Schwalenstöcker B, Soyal SM, et al. PGC-1α is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis. Hum Mol Genet. 2013.
Acknowledgements: The work is funded by a pilot grant from the Thierry-Latran-Foundation (to PW) and by the Helmholtz Virtual institute "RNA dysmetabolism in Amytrophic Lateral Sclerosis and Frontotemporal Dementia" (to AW, LD and PW).

P.78: DIFFERENTIAL EXPRESSION OF NEUROTROPHIC FACTORS IN SPINAL CORD AND MUSCLE OF MUTANT SOD1 MICE *Kefalakes E., Thau-Habermann N., Jekel M., Grothe C., Petri S. Ekaterini Kefalakes, Department of Neurology, Hannover Medical School Marco Jekel, Department of Neurology, Hannover Medical School Nadine Thau-Habermann, Department of Neurology, Hannover Medical School Claudia Grothe, Department of Neuroanatomy, Hannover Medical School Susanne Petri, Department of Neurology, Hannover Medical School*

Neurotrophic growth factors have been described as key players in the development, function and maintenance of motor neurons and are thus implied in the pathogenesis of amyotrophic lateral sclerosis (ALS). We previously described that the knock-out of fibroblast growth factor-2 (FGF-2) in the SOD1G93A transgenic mouse model of ALS results in a prolonged survival attributed to a delay in disease onset. These neuroprotective effects are, at least, partially associated with compensatory up-regulation of other growth factors (CNTF and GDNF).
Therefore, our aim now is to characterize the expression patterns of FGF-2 and its dependent growth factors, as well as growth-factor activated signaling cascades in ALS transgenic mice.
Gene expression analyses in mutant SOD1G93A mice were performed to determine the levels of growth factor, growth factor receptor and their consecutive signaling molecule mRNA expression at different time points, in order to elucidate their roles during disease progression. For this purpose, spinal cord and muscle homogenates of SOD1 (B6.Cg-Tg(SOD1*G93A)1Gur/J) and wild type mice were analyzed at day 90, 120 and 150 by quantitative real-time PCR.
Growth factors and several players of respective signaling cascades were significantly up- or downregulated in both spinal cord and muscle samples in a disease stage- dependent manner. Prominently, FGF-2, CNTF and GDNF mRNA were significantly increased in the spinal cord of SOD1G93A animals, which correlates with studies in human sporadic ALS tissue.
Detailed characterization of neurotrophic factor spatiotemporal expression patterns including cell specific analyses of motor neurons and glial cells as well as the evaluation of the dependent signaling pathways will further elucidate disease-specific dysregulations and their therapeutic potential.

SOCIAL EVENTS

Visit to the Old Library and Historic Book of Kells

The Book of Kells was written by Irish monks in 800AD on the Scottish island of Iona, and transported to Kells in Ireland for safe keeping during the Viking raids.
It has been on display in the Old Library at Trinity College Dublin from the mid 19th century.

Opening Hours: **Monday-Saturday 9.30-17.00**
Sunday 09.30-16.30
See Map page 102

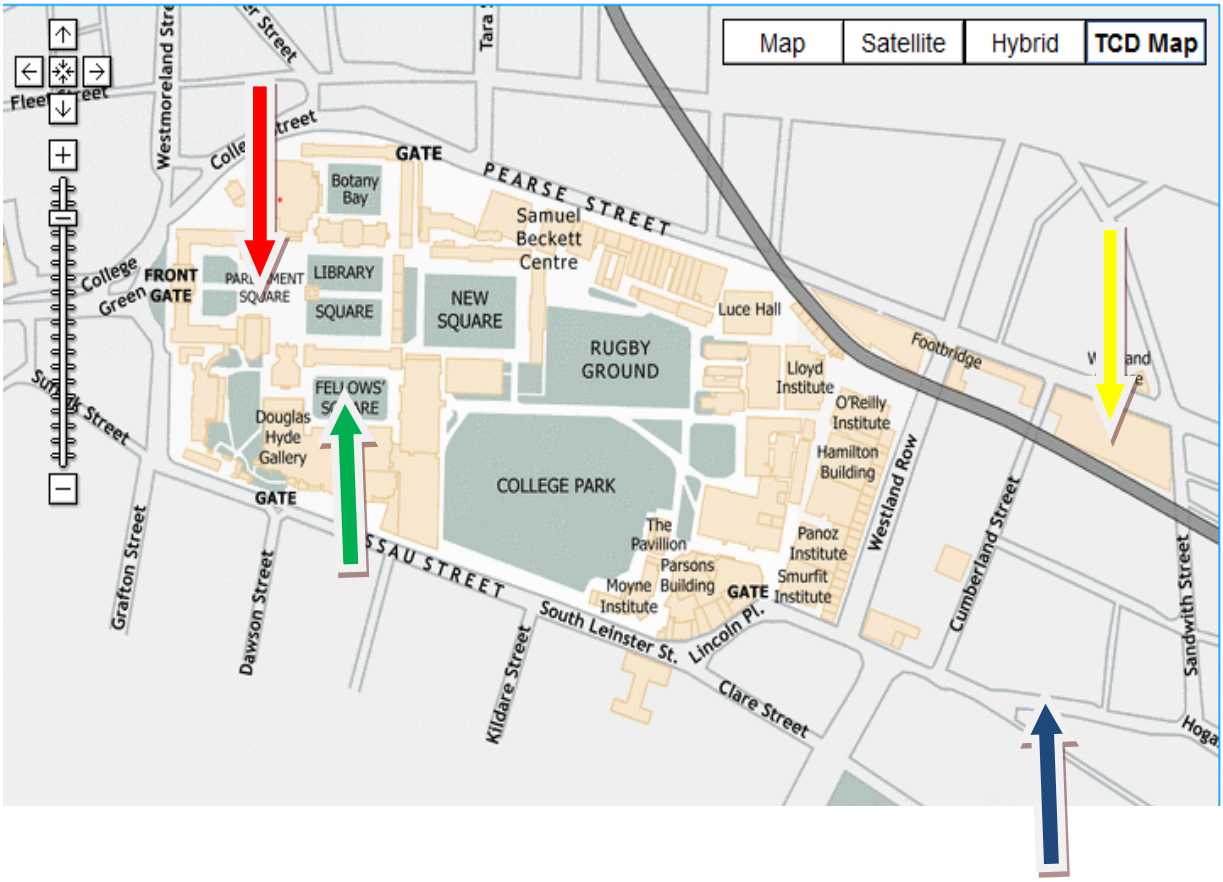
The Old Jameson Distillery
Opening hours: Monday–Sunday 09.00-18.00.
Last tour at 17.15
https://bookings.jamesonwhiskey.com/ `

The Guiness StoreHouse
Monday-Sunday: 09.30-5.00
http://www.guinness-storehouse.com

Bus Tours - Dublin Sight Seeing
Hop on, Hop off
http://www.dublinsightseeing.ie/citytour.aspx

National Gallery of Ireland
Merrion Square West, Dublin 2
Opening Hours:
Monday–Saturday 9.30am – 5.30pm
Thursday 9.30am - 8.30pm
Sunday 11am - 5.30pm

MAP OF CAMPUS



Yellow Arrow:	Biomedical Sciences Insitute (Conference)
Red Arrow:	Dining Hall (Gala dinner, Saturday evening)
Blue Arrow:	Alexander Hotel, Fenian Street (Cytokinetics Investigator Reception, Thursday evening)
Green Arrow:	Old Library, Book of Kells

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