MESSAGE ROM THE ORGANIZERS

Dear participant of the 10th Meeting of ENCALS,

CÉAD MÍLE FÁILTE GO BAILE ATHA CLIATH!

We welcome you to Trinity College Dublin for the 10th 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 new therapeutics, novel mechanisms of disease, genetics and clinical phenotyping. We are delighted to have as our guests three of the most eminent of ALS researchers from the United States, Dr. Teepu Siddique, Dr. Bob Brown and Dr. Jeff Rothstein. We also welcome our distinguished plenary speakers from Europe, Dr. Abrahams, Dr. Boillé and Prof. Talbot. 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.

This year, we have decided to hold a debate, in which audience participation will be actively encouraged. The deliberately controversial topic is about the longer-term utility of the SOD1 mouse as an appropriate model of ALS.

We expect a lively exchange – don't be shy!

We gratefully acknowledge the support from Biogen Idec, and administrative support from Trinity College Dublin and Research Motor Neurone.

We thank the members of the scientific committee, Petra Berk from the University of Utrecht for her excellent administrative support of ENCALS and Dominique Plant, who have 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

CONTENT

PROGRAMME OVERVIEW

Friday, 25th May 2012

08.30 - 11.30	Workshop on neurophysiology MUNIX and related measures
11.30 - 13.45	Registration and lunch
13.45 - 14.45	Opening Session: Latran Guest Speaker
14.45 – 16.15	Session 1: Applied Epidemiology & Deep Phenotyping
16.15 - 16.30	Coffee
16.30 – 18.00	Session 2: Therapeutic Targets in ALS
18.00 – 19.30	Guided Poster Tour with Cheese and Wine Reception

Saturday, 26th May 2012

08.30 – 09.00	Session 3: Disease Mechanisms in ALS and Related Neurodegenerations
10.30 - 11.00	Coffee
11.00 – 12.30	Session 4: Cognition and Imaging
12.30 - 14.00	Lunch
14.00 – 15.30	Session 5: Disease Mechanisms, Glia
15.30 - 15.50	Coffee
15.50 –17.00	Session 6: Debate
17.00 - 17.30	ENCALS business meeting
17.30 - 19.00	Guided Tour Posters with wine reception
19.00 - 20.00	Optional Tour of Campus
20.00	Dinner in the Dining Hall College Square

Sunday, 27th May 2012

08.00 - 08.30	ENCALS C9orf72 Consortium
08.30 – 09.00	ENCALS Young Investigator Award
09.00 – 11.30	Session 7: Genetics and Genomics
11.30 – 12.00	Coffee
12.00 – 13.00	Session 8: New Developments
13.00	Meeting Conclusion

Friday, 25th May

08.30 – 11.30 **Workshop on Neurophysiology MUNIX and Related measures** B2.72-2.73-2.74

11.30 - 13.45 Registration and lunch Hallway, Ground Floor

13.45 - 14.45 Opening Session Stanley Quek Theatre B1
Leonard H. van den Berg

Invited Speaker: Latran Foundation: *Dr. Teepu Siddique (USA)*
"Cause to mechanism: the molecular funnel of neurodegeneration"

14.45 – 15.15 **Oral Session 1: Applied Epidemiology & Deep Phenotyping**
Chair: Adriano Chio, Markus Weber

Plenary Speaker: Orla Hardiman (Ireland)

15.15 - 15-35 OS1.1: Motor Unit Number Index (MUNIX): longitudinal measurements in ALS patients in a multicentre trial

C. Neuwirth (Switzerland)

15.35 - 16.55 OS1.2: Symptom development in patients with motor neuron disease

R. Walhout (Netherlands)

16.55 - 16.15 OS1.3: An exposome wide association study in ALS

P. van Doormaal (Netherlands)

16.15 - 16.30 **Coffee**

16.30 – 17.00 **Oral Session 2: Therapeutic Targets in ALS**
Chair: Francois Salachas, Pam Shaw

Plenary Speaker: Jeff Rothstein (USA)

17.00 - 17.15 OS 2.1: Dexpramipexole effects on functional decline in ALS patients in a Phase II study: subgroup analysis of demographic and clinical characteristics

S.A. Rudnicki (Biogen Idec, Cambridge MA)

17.15 - 17.30 OS2.2: Prolonged survival and milder impairment of motor function in the SOD1 mouse model devoid of FGF-2

N. Thau (Germany)

17.30 - 17.45 OS2.3: EphA4 inhibition rescues the motor axon phenotype in a zebrafish model for ALS and SMA

L. Schoonaert (Belgium)

17.45 - 18.00 OS2.4 :A Phase 2-3 Trial of Olesoxime in Subjects with Amyotrophic Lateral Sclerosis

Olesoxime for ALS Study Group (France)

18.00 - 19.30 **Guided Poster Tour with Cheese and Wine Reception**

Knowledge Exchange (Level 2)

Saturday, 26th May

08.30 – 09.00 **Oral Session 3: Disease Mechanisms in ALS and Related Neurodegenerations**

Chairs: Jochen Prehn, Caterina Bendotti

Plenary Speaker: Kevin Talbot (UK)

09.00 - 09.15 OS3.1: Elevated mitochondrial calcium extrusion disturbs ERMCC dynamics in G93A hSOD1 motor neurons

J. Lautenschlaeger (Germany)

09.15 - 09.30 OS3.2: PLCdelta1 knockout prolongs survival of ALS mice

K.A. Staats (Belgium)

09.30 - 09.45 OS3.3: Molecular mechanisms of Golgi pathology in progressive motor neuronopathy

S. Bellouze (France)

09.45 - 10.00 OS3.4: Elevated PGC-1a activity sustains muscle function throughout disease in a model of familial ALS

P. Parone (USA)

10.00 - 10.15 OS3.5: Utilizing a SOD1 zebrafish model of ALS to dissect neuronal circuits involved in ALS and in drug screening

T. Ramesh (UK)

10.15 - 10.30 OS3.6: Altered expression and activity of stearoyl-CoA desaturase 1 in amyotrophic lateral sclerosis

F. Schmitt

10.30-11.00 **Coffee**

11.00-12.30 **Oral Session 4: Cognition and Imaging**

Chair: Julian Grosskreutz, Martin Turner

Plenary Speaker: Sharon Abrahams (SCOTLAND)

11.30 - 11.45 OS4.1: Evidence for Subtypes within the Cognitive Continuum: A Population-Based Longitudinal Study

M.Elamin (Ireland)

11.45 - 12.00 OS4.2: Quantitative analysis of corticospinal tract hyperintensity in ALS

J. Fabes (UK)

12.00 - 12.15 OS4.3: Amyotrophic lateral sclerosis spreads along structural brain connections

E. Verstraete (Netherlands)

12.15 - 12.30 OS4.4: Grey matter correlates of clinical variables in Amyotrophic Lateral Sclerosis– a neuroimaging study

P. Bede (Ireland)

12.30-14.00 **Lunch** **Alexander Hotel**

14.00 – 15.30 **Oral Session 5: Disease Mechanisms, Glia**

Chair: Vincenzo Silani, Ludo van den Bosch

Plenary Speaker: Severine Boillee (FRANCE)

14.30 - 14.50 OS 5.1: Expression and function of system Xc- and microglial glutamate in ALS Models

L. Mesci Pinar (France)

14.50 - 15.10 OS 5.2: Altered intracellular calcium signaling correlates with Astrocyte degeneration in ALS

D. Rossi (Italy)

15.10 - 15.30 OS 5.3: Neuro-muscular junctions in Co-cultures of hiPSCs-derived Motoneurons and Myotubes

M. Stockman (Germany)

15.30 - 15.50 **Coffee**

15.50 – 17.00 **Oral Session 6: Debate**

"Has the SOD1 animal model passed its sell-by date?"

(Audience Participation Strongly Encouraged)

Moderator: Leonard van den Berg

Speaker: Prof. Pamela J. Shaw (University of Sheffield)

Speaker: Prof. Wim Robberecht (Catholic University Leuven)

17.00 - 17.30 ENCALS Business Meeting

17.30 - 19.00 **Guided Tour Posters with wine reception**

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

Sunday, 27th May

08.00 -08.30 **ENCALS C9orf72 Consortium Meeting**
(*all welcome)*

Moderator: Ammar Al Chalabi

08.30 - 09.00 **ENCALS YOUNG INVESTIGATIOR AWARD**

09.00 – 11.30 **Oral Session 7: Genetics and Genomics**
Chair: Dan Bradley, Jan Veldink

Plenary Speaker: Bob Brown (USA)

09.30 - 09.45 OS 7.1: FUSopathy in cells and transgenic mice expressing an aggregation prone form of human protein

V. Buchman (UK)

09.45 - 10.00 OS 7.2: ALS-associated mutant VAPBP56S perturbs Ca2+ homeostasis to disrupt axonal transport of mitochondria

G. Mórotz (UK)

10.00 - 10.15 OS 7.3: Gene expression profiling of lymphoblastoid cells from C9ORF72 related amyotrophic lateral sclerosis

J. Cooper-Knock (UK)

10.15 - 10.30 OS7.4: C9ORF72 hexanucleotide repeat expansion in Italian ALS patients

N. Ticozzi (Italy)

11.00 - 11.15 OS7.5: UNC13A influences survival in an Italian population-based series

A. Calvo (Italy)

11.15 - 11.30 OS7.6: PGC-1 alpha as a genetic modifier in experimental ALS

B. Schwalenstöcker (Germany)

11.30 – 12.00 **Coffee**

12.00 – 13.00 **Oral Session 8: New Development**

Chair: Orla Hardiman, Leonard van den Berg

12.00 - 12.15 OS8.1: Executive Dysfunction in ALS relates to changes in white matter integrity in the frontal lobes

L. Pettit (Scotland)

12.15 - 12.30 OS8.2: Comparison of the King's amyotrophic lateral sclerosis staging system with the revised amyotrophic lateral sclerosis functional rating scale

R. Balendra (United Kingdom)

12.30 - 12.45 OS8.3: Systemic delivery of angiogenin protein for the treatment of ALS

A. T. Behan (Ireland)

12.45 - 13.00 OS8.4: Identification of neurotrophic factors for ALS-relevant motor neuron subsets by a novel FACS-based approach

D. Buttigieg (France)

13.00 Meeting Conclusion

POSTER SESSIONS

Posters will be displayed throughout the meeting. Knowledge Exchange (Level 2)

Poster Session I
Friday, 25/05, 18.00 – 19.30 (Chairs: Jesus Mora and Susanne Petri)

CLINICAL

P.01: SPINAL MRI ATROPHY STUDY IN AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY

EL MENDILI, M-M; Cohen-Adad, J; Morizot-Koutlidis, R; Lenglet, T; Serge, R; Benali, H; Pradat, P-F (Laboratoire d\\\'imagerie fonctionnelle (LIF) U.678 INSERM / UMR-S UPMC)

P.02: REPORTING BIOMAKER – DEVELOPMENT IN ALS PATIENTS TREATED WITH G-CSF MOBILIZED STEM CELLS

Prof. Ulrich Bogdahn (University of Regensburg - Department of Neurology)Jennifer Rösl, Andrei Khomenko, Dobri Baldaranov, Jochen Grassinger, Verena C. Haringer1, Katja Kollewe **, Renata Schreck, Susanne Petri **, Reinhard Dengler **, Albert Ludolph , Bernhard Kaiser, Michael Deppe***, Gerhard Schuierer#, Wilhelm Schulte-Mattler, Ulrich Bogdahn - Regensburg, San Diego, Ulm, Münster, Hannover*

P.03: SUSCEPTIBILITY MRI REVEALS ALTERED MAGNETIC BEHAVIOUR IN ALS-RELATED WHITE MATTER DAMAGE

Hartung, V; Prell, T; Tietz, F; Ilse, B; Reichenbach, J; Schweser, F; Witte, OW; Grosskreutz, J (Hans Berger Department of Neurology, University Hospital Jena, Germany)

P.04: DISTRIBUTION OF ADIPOSE TISSUE IN PATIENTS WITH ALS ASSESSED BY AUTOMATIC WHOLE BODY MRI ANALYSIS

H.-P. Müller1, E. Lindauer1, L.Dupuis1, H. Neumann2, J. Kassubek1, A.C. Ludolph1 (1Dept of Neurology, Univ. Ulm, Germany, 2Inst of Neural Information Processing, Univ. Ulm, Germany)

P.05: MONOCLONAL GAMMOPATHY IN THE FULL SPECTRUM OF MOTOR NEURON DISORDERS AND MULTIFOCAL MOTOR NEURON DISORDERS AND MULTIFOCAL MOTOR NEUROPATHY

L. Vlam, MD,1 S. Piepers, MD, PhD,1 N.A. Sutedja, MD, PhD,1 B.C. Jacobs MD, PhD,2 A.P. Thio-Gillen,2 J. H. Veldink, MD, PhD,1 E.A. Cats, MD, PhD,1 F. Brugman, MD, PhD,1 N.C. Notermans MD, PhD,1 R.I. Wadman MD,1 W.-L. van der Pol MD, PhD,1 L.H. van den Berg MD, PhD.1

P.06: IMMUNOLOGICAL STATUS OF PATIENTS WITH ALS

Zorica Stevic, Clinic of Neurology, School of Medicine, Belgrade, Serbia

P.07: ELECTRONIC PATIENT- REPORTED OUTCOME TO EVALUATE PHYSIOTHERAPY TREATMENT IN ALS

Robert Meyer; Christoph Münch; Andre Maier; Theresa Holm; Laura Steinfurth; Thomas Meyer (Department of Neurology, Charité-University Hospital, Campus Virchow-Klinikum, Augustenburger Platz)

P.08: ILLNESS BURDEN IN PATIENTS WITH ALS AND THEIR CAREGIVERS: A WEB-BASED SURVEY

Paul Wicks[1], Massagli MP[1], Leigh Ann White[2] [1] PatientsLikeMe Inc., 155 Second Street, Cambridge MA 02141

[2] Biogen Idec, 133 Boston Post Road, Weston MA 02493

P.09: SELF-ASSESSMENT OF THE DAILY FOOD INTAKE IN ALS VIA AN APPLICATION

T. Holm, A. Maier, L. Steinfurth, J. Leimeister, A. Prinz, P. Linke, R. Meyer, C. Münch, T. Meyer (Charité University Hospital)

P.10: WEB-BASED SELF-ASSESSMENT OF DYSPNOEA IN ALS

André Maier (Charité - Universitätsmedizin Berlin)

P.11: TIME TO GENERALIZATION AS A PREDICATOR OF PROGNOSIS IN ALS *Tortelli R, Zoccolella S, Cortese R, D'Errico E, Capozzo R, Leo A, Simone IL, Logroscino G. (Department of Neurosciences and Sense Organs, University of Bari, Italy)*

P.12: WEIGHT LOSS IN ALS: REASONS, IMPACT ON QUALITY OF LIFE AND BENEFIT OF HIGH CALORIE SUPPLEMENTS

Körner S, Hendricks M, Kollewe K, Dengler R, Petri S

P.13: PHRENIC NERVE STUDIES PREDICT SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS

Susana Pinto, Anabela Pinto, Mamede de Carvalho Translational and Clinical Physiology Unit, Institute of Molecular Medicine-Faculty of Medicine, University of Lisbon, Portugal

P.14: GAMMA-SYNUCLEIN PATHOLOGY IN ALS

Dr Owen Peters (Cardiff University)

P.15: IS THE FRONTAL ASSESSMENT BATTERY RELIABLE IN ALS PATIENTS?

Joost Raaphorst (Academic Medical Centre, Amsterdam, The Netherlands)

P.16: LANGUAGE AND COGNITIVE IMPAIRMENTS IN BULBAR-ONSET AMYOTROPHIC LATERAL SCLEROSIS

Sarafov S, Raycheva M, Mehrabian S, Tournev I, Traykov L (University Hospital "Alexandrovska, Sofia, Department of Neurology)

EPIDEMIOLOGY

P.17: WHY DOES THE AGE AT ONSET OF AMYOTROPHIC LATERAL SCLEROSIS DIFFER BETWEEN COUNTRIES?

Susan Byrne, Iain Jordan, Marwa Elamin, Orla Hardiman (Trinity College Dublin, Ireland)

P.18: THE ALS REGISTER SWABIA: EPIDEMIOLOGY AND RISK FACTORS IN SOUTHERN GERMANY
A. Rosenbohm, D. Rothenbacher , G. Nagel, Albert C. Ludolph (Departments of Neurology and Medical Biometry and Epidemiology, Ulm University, Germany)

P.19: MEDICATION USE, PAST MEDICAL HISTORY AND THE RISK OF ALS: A POPULATION-BASED CASE-CONTROL STUDY
Meinie Seelen, MD[1]; Perry TC van Doormaal, MD[1]; Margot HJ Roozekrans, MD[1]; Mark HB Huisman, MD[1]; Sonja W de Jong, MD[1]; H Jurgen Schelhaas, MD, PhD[2]; Anneke J van der Kooi, MD, PhD[3]; Marianne de Visser, MD, PhD[3]; Jan H Veldink, MD, PhD[1]; Leonard H van den Berg, MD, PhD[1]. (Utrecht, Holland)

P.20: MAY HYPERTENSION HAVE PROTECTIVE EFFECTS IN ALS? RESULTS OF A POPULATION-BASED STUDY
Ilardi, Calvo, Moglia, Canosa, Cammarosano, PARALS, Mazzini, Mora, Chiò (CRESLA, Department of Neuroscience, University of Turin)

P.21: PREVALENCE OF IMMUNE-RELATED COMORBIDITY AMONG ALS PATIENTS IN A U.S. HEALTH INSURANCE CLAIMS DATABASE
JR Williams[1], DA Kerr[1], and W Farwell[1] [1]Biogen Idec, Cambridge, MA, USA

P.22: PREVALENCE OF CARDIOVASCULAR AND CEREBROVASCULAR RISK FACTORS AND COMORBIDITY AMONG ALS PATIENTS IN A U.S. HEALTH INSURANCE CLAIMS DATABASE
JR Williams1, DA Kerr1, and W Farwell1 (1Biogen Idec, Cambridge, MA, USA)

P.23: EUROMOTOR
P.Berk Utrecht*, Holland*

POSTER SESSION II
Saturday, 26/05, 17.30-19.30 (Chairs: Albert Ludolph and Karen Morrison)

GENETICS

P.24: SCREENING FOR RARE VARIANTS IN THE CODING REGION OF ALS ASSOCIATED GENES AT 9p21.2 AND 19p13.3
Max Koppers (University Medical Center Utrecht)

P.25: ANALYSIS OF THE H63D POLYMORPHISM IN HFE AS SUSCEPTIBILITY FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS
Mr. Wouter van Rheenen (University Medical Center Utrecht)

P.26: IDENTIFYING NOVEL RISK AND PROTECTIVE ALS VARIANTS THROUGH TARGETED RESEQUENCING
Kevin Kenna, Russell McLaughlin, Orla Hardiman, Dan Bradley (Trinity College Dublin)

P.27: IDENTIFY-BY-DESCENT MAPPING REVEALS GENOMIC LOCI THAT MAY HARBOUR MULTIPLE RARE ALS-CAUSING VARIANTS
Russell L McLaughlin, Kevin P Kenna, Dan G Bradley, Orla Hardiman (Trinity College Dublin)

P.28: HAS THE TIME NOW COME FOR A REVISION OF THE ALS GENE CLASSIFICATION SYSTEM?

Rubika Balendra, Ammar Al-Chalabi (Department of Clinical Neuroscience, King\'s College London)

P.29: ALS-CAUSING MUTANT TDP-43 VARIANTS AND THEIR EFFECT ON PROTEIN DEGRADATION PATHWAYS

H.Wolf(1), A.Besemer(1), M.Stark(1), I.Drechsler(1), H.Witan(1), JP.Julien(2), C.Behl(1), A.Clement(1) (1)Institute for Pathobiochemistry,University Medical Center,Johannes Gutenberg University Mainz,Ger)

P.30: P2RX7 POLYMORPHISMS AND SUSCEPTIBILITY TO ALS IN AN ITALIAN POPULATION: PRELIMINARY RESULTS

Michele Benigni[1], Claudia Ricci[1], Stefania Casali[1], Giannini Fabio[1], Cinzia Volonté[2], Stefania Battistini[1]

[1]Department of Neurological, Neurosurgical, and Behavioral Sciences, University of Siena, Siena, Italy

[2]Cellular Biology and Neurobiology Institute, CNR Fondazione Santa Lucia, Rome, Italy

P.31: SYSTEMIC DEPLETION OF SCD1 PROMOTES ACCELERATED MOTOR FUNCTION RECOVERY FOLLOWING NERVE INJURY

G Hussain, F Schmitt, F Rene, A Henriques, J-L Gonzalez De Aguilar, J-P Loeffler (Inserm, Umrs692, Université de Strasbourg, Strasbourg, France

P.32: GENETICS OF ALS IN ITALY: RESULTS OF A POPULATION-BASED STUDY

[1]Calvo A, [5]Restagno G, [5]Brunetti M, [5]Ossola I, [1]Moglia C, PARALS, [3]Corrado L, [3]D'Alfonso S, [4]Mazzini L, [2]Mora G, [1]Chiò A, [1]Als Center, Department of Neuroscience, University of Troino, Italy

P.33: CGG-REPEAT EXPANSION IN FMR1 IS NOT ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS

Ewout J N Groen[a,b], Wouter van Rheenen[a], Max Koppers[a,b], Perry T C van Doormaal[a], Lotte Vlam[a], Frank P Diekstra[a], Dennis Dooijes[c], R Jeroen Pasterkamp[b], Leonard H van den Berg[a], Jan H Veldink[a]

P.34: ASSOICATION OF FUS WITH THE CYTOSKELETAL PROTEINS TBCB AND DCTN1

Stefan Putz (University Hospital Ulm)

P.35: AMYOTROPHIC LATERAL SCLEROSIS CARRYING EXPANSION OF C9ORF72 WITH OBSESSIVE-COMPULSIVE DISORDER AT ONSET

Moglia C, Calvo A, Canosa A, Ilardi A, Bersano E, Manera U, Restagno G, Chio' A ALS center, Department of Neuroscience, University of Torino, Italy

P.36: FASTER DISEASE PROGRESSION IN fALS LINKED TO C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSIONS

A. Hübers (1), A. Volk (2), C. Kubisch (2), N. Marroquin (2), A.C. Ludolph, J.H. Weishaupt (1) ((1) Department of Neurology, (2) Department of Human Genetics, University of Ulm, Germany)

P. 37: THE MOLECULAR BASIS OF ALS IN TURKEY

Ozoguz A1, Bilguvar K2, ParmanY3, Deymeer F3, Oflazer P3, Koc F4, Gunel M2 and Basak A N1 1Bogazici University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey 2Yale University Medical School, Department of Neurosurgery, Gunel Laboratory, New Haven, USA 3Istanbul University, Istanbul Medical School, Neurology Department, Istanbul, Turkey 4Cukurova University, Medical School, Neurology Department, Adana, Turkey

P.38: ATXN2 AND ITS NEIGHBOURING GENE SH2B3 BOTH MODULATE THE ALS RISK IN THE TURKISH POPULATION

Lahut S1, Ömür Ö1, Uyan Ö1, Ağım Z S1, Özoğuz A1, ParmanY2, Deymeer F2, Oflazer P2, Koç F3, Özçelik H4, Auburger G5 and Başak A N11Boğaziçi University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey 2Istanbul University, Istanbul Medical School, Neurology Department, Istanbul, Turkey 3Çukurova University, Medical School, Neurology Department, Adana, Turkey 4University of Toronto, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Department of Laboratory Medicine and Pathobiology, Toronto, ON, Canada 5Goethe University, Experimental Neurology, Frankfurt am Main, Germany

***P.39*: IN-SILICO AGGREGATION PROFILE ANALYSIS OF UBQLN2 MUTATIONS IN TURKISH FALS PATIENTS** Uyan *O1, Agim ZS1, Ozoguz A1, Keskin O2, Basak AN1 1Bogazici University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey 2 Koc University, College of Engineering, Chemical and Biological Engineering, Istanbul, Turkey*

MODELS

P.40: EFFECT OF FUMARIC ACID ESTERS ON THE HIF-1alpha MEDIATED RESPONSE

Diana Wiesner, Judith Eschbach, A.C. Ludolph, Anke Witting, Luc Dupuis (University of Ulm, Germany)

P.41: UPREGULATION OF PDI IN ALS MICROGLIA AND ER STRESS DEPENDENT ACTIVATION OF NADPH OXIDASE

Ms. Merja Jaronen (Dept. of Neurobiol., A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland)

P.42: EFFECT OF PPARs AND THEIR CO-ACTIVATORS ON THE ALTERNATIVE ACTIVATION OF MICROGLIA

Hanna Bayer, Anne Buttgereit, Diana Wiesner, Patrick Weydt und Anke Witting (Department of Neurology, Ulm University, Germany)

P.43: KINESINS EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF HUMANS AND TRANSGENIC MICE WITH hSOD1G93A

Magdalena Kuzma-Kozakiewicz, Agnieszka Chudy, Ewa Usarek, Beata Gajewska, Anna Baranczyk-Kuzma (Department of Neurology, Department of Biochemistry, Medical University of Warsaw, Poland)

P.44: HDAC6 INHIBITION RESTORES THE PHENOTYPE OF NEW MOUSE MODLES OF CHARCOT-MARIE-TOOTH DISEASE

Constantin D'Ydewalle (Vesalius Research Center, Laboratory of Neurobiology, VIB, KU Leuven, Leuven, Belgium)

P.45: GENDER-SPECIFIC MECHANISM OF SYNAPTIC IMPAIRMENT AND ITS PREVENTION BY G-CSF IN A MOUSE MODEL OF ALS

Eveliina Pollari (Dept. of Neurobiol., A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland)

P.46: TDP-43 DYSFUNCTION CAUSE SYNAPTIC DEFICITS AND SUBSEQUENT AGE-RELATED NEURODEGENERATION IN DROSOPHILA

Danielle C. Diaper[1,7], Yoshitsugu Adachi[1,7], Ben Sutcliffe[2], Dickon M. Humphrey[1], Chris Elliott[3], Triona Fielding[1], Mubarik Burki[1], Zoe N. Ludlow[1], Lies Vanden Broeck[4], Bart Dermaut[4,5], Patrick Callaerts[4], Ammar Al-Chalabi[6], Christopher E. Shaw[6], Iain Robinson[2] & Frank Hirth[1]

P.47: POTENTIAL EFFECTS OF LOW-FREQUENCY MAGENTIC FIELDS (LF-MFs) ON AMYOTROPHIC LATERAL SCLEROSIS

Martina Liebl (1), Andreas Horn (1), Blanka Pophof (2), Christian Behl (1), Albrecht M. Clement (1) (Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg University Mainz)1. Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg University Mainz, Germany 2. Federal Office for Radiation Protection, Salzgitter, Germany

P.48: COMMON EARLY ALTERATIONS IN LUMBAR MOTONEURONS OF SOD1G93A AND SOD1G85R JUVENILE MICE

Jacques Durand, Anton Filipchuk, Arnaud Pambo Pambo, Sylvie Liabeuf, Cecile Brocard, J.P. Gueritaud (CNRS, UMR 7289, Aix Marseille Université, Institut de Neuroscience de la Timone, Marseille, France)

P.49: NEGATIVE RESULTS: CITICOLINE IS NOT PROTECTIVE IN THE SOD1 (G93A) MOUSE MODEL OF ALS

Dr. Sarah Knippenberg, Thomas Skripuletz, Nadine Thau, Klaus-Jan Rath, Reinhard Dengler, Martin Stangel, Susanne Petri

P.50: USE OF MAGNETIC RESONANCE IMAGING TO MONITOR DISEASE PROGRESSION IN ANIMAL MODELS OF FAMILIAL ALS

Ilaria Caron (Mario Negri Institute for Pharmacological Research), Micotti E., Plebani, L., Merlino G. and Bendotti C

P.51: PRE-SYMPTOMATIC NEUROINFLAMMATORY INSIGHTS FROM MULTIMODAL MRI IN THE SOD1 MURINE MODEL OF ALS

Evans MC, Stolp HB, Anthony D, Talbot K, Sibson N, Turner MR (University of Oxford)

P.52: MOBILIZATION OF HEMATOPOIETIC BONE MARROW STEM CELLS BY G-CSF IS NOT PROTECTIVE WHEN INDUCED IN SYMPTOMATIC SOD1G93A MOUSE MODELS

Bendotti C., Caron I., Ferrara G., Merlino G., Plebani L. Dept. Neuroscience, Mario Negri Institute for Pharmacological Research, Milano,Italy

P.53: IS DEFECTIVE MITOCHONDRIAL Ca2+ STORAGE IS A PRIMARY CONTRIBUTOR TONEURONAL DEGENERATION?

P. Parone Department of Cell Biology, University of Geneva, Geneva, Switzerland.

P.54: SOD1 mRNA ARE SEQUENCES AS NOVEL TARGETS FOR ELAV-MEDIATED POST-TRANSCRIPTIONAL MODULATION: A NEW CLUE FOR ALS PATHOGENESIS?

Pamela Milani (1, 2), Marialaura Amadio (3), Stella Gagliardi (1), Alessia Pascale (3), Cristina Cereda (1)
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P.55: SENSING ION CHANNELS (ASICS) CONTRIBUTE TO MOTONEURON DEGENERATION IN AN ANIMAL MODEL OF ALS

by Aine T Behan#, Bridget Breen#, Ina Woods, Karen Coughlan, Mollie Mitchem, Jochen H.M.Prehn (Department of Physiology and Medical Physics, Centre for the Study of Neurological Dis orders, RCSI) Dublin Ireland

P.56: ANDROGENIC/ ANABOLIC STEROID DYSREGULATION AS RISK FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS

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ABSTRACTS

OS1.1: MOTOR UNIT NUMBER INDEX (MUNIX): LONGITUDINAL MEASUREMENTS IN ALS PATIENTS IN A MULTICENTRE TRIAL

C. Neuwirth (Muskelzentrum / ALS clinic, Kantonsspital St.Gallen, Switzerland)

Background: Sensitive outcome measures are sorely needed in ALS clinical trials. In single center studies MUNIX has shown to be a reliable and feasible motor unit number estimation (MUNE) method in ALS patients. To evaluate MUNIX as an outcome measure/biomarker in a multicenter natural history study of ALS subjects.
Methods: In 3 participating centres 24 ALS patients (12 limb, 12 bulbar onset) were recruited. MUNIX measurements were performed every 3 months in 6 muscles: biceps, abductor digiti minimi, abductor pollicis brevis, tibialis anterior, abductor hallucis and extender digitorum brevis. 9 months data were available for 11 patients (8 limb, 3 bulbar onset). Results: Initial mean MUNIX value over all muscles was 88, mean CMAP amplitude 5.8. For limb onset patients, initial MUNIX was 80 and in bulbar onset 95. MUNIX dropped by approximately 40% from baseline after 9 months. Mean decline of MUNIX was 10 every 3 months. Decline of mean CMAP amplitude from baseline was 0.6 mV every 3 months. Further collection of data is ongoing.
Conclusion: Preliminary data show that MUNIX might be an adequate electrophysiological outcome measure/biomarker in longitudinal multi-center ALS studies. One outstanding advantage is the possibility to examine multiple muscles in an adequate amount of time. Further data collection is in progress and statistical analysis will be performed to compare this method with other established markers like the ALSFRS.

OS1.2: SYMPTOM DEVELOPMENT IN PATIENTS WITH MOTOR NEURON DISEASE

R. Walhout; E. Verstraete; M.P. van den Heuvel; J.H. Veldink; L.H. van den Berg (Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht,)

Mechanisms underlying the progressive loss of motor neurons in amyotrophic lateral sclerosis (ALS) are still unknown. Recently, it has been suggested that spread of disease is guided by neural connections. The pattern of symptom development in motor neuron diseases might shed light on the underlying neuron degeneration. While many studies focused on symptoms of ALS, reports on pure upper or lower motor neuron disease are relatively scarce. Therefore, we investigated symptom development within the spectrum of motor neuron diseases, looking for patterns of disease spread. Six hundred patients with ALS, PMA and PLS were invited to complete a questionnaire concerning development of symptoms over time. A binomial test was used to investigate whether spread of symptoms from site of onset to subsequent body regions was random. Timing of symptom development was evaluated by Kaplan Meier analysis. Two hundred fifty-four ALS patients, 100 PMA and 116 PLS patients returned the questionnaire (81%). Disease progression in these patients was characterized by a non-random spread of symptoms; ALS patients reported significantly more often symptom spread to the opposite limb following unilateral limb onset ($p < 0.01$). The same pattern was seen in patients with PMA and for PLS patients in the lower limbs. Concerning the timing of disease progression, symptoms were found to spread faster to the opposite limb, followed by the other limbs and lastly by the bulbar region. Preferred spread of symptoms from one limb to the opposite limb is a uniform feature of motor neuron disease, irrespective of upper or lower motor neuron involvement. Our results in PLS patients support disease progression along the corpus callosum, rather than cortical spread over the motor cortex. The pattern of symptom development in motor

neuron disease suggests that motor neuron degeneration is guided by structural and functional connectivity.

OS1.3: AN EXPOSOME WIDE ASSOCIATION STUDY IN ALS

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Background: Environmental factors are believed to play an important role in the development of sporadic amyotrophic lateral sclerosis (ALS). It is unknown how these factors interact or confound each other's effect. Objectives: To study the total of exogenous factors (the exposome) and their interaction on the development of ALS, correcting for multiple testing.

Methods: A large population-based case-control study was conducted between January 2006 and December 2011 in the Netherlands. 735 incident patients and 2027 controls filled in questionnaires about multiple exogenous factors. Logistic regression was used to calculate the association of these factors with ALS. Data reduction with principal components was performed. In addition, we looked for differences in the correlation structure between risk factors between ALS and controls (differential co-exposition analysis).

Results: Not drinking alcohol, no lipid modifying medication, premorbid weight loss, increased intake of saturated fat, increased intake of total fat, reduced intake of fibers, reduced intake of plant proteins, reduced intake of flavonoids, premorbid BMI and increased leisure time physical activities were all significantly associated with ALS. Principal component analysis showed 6 components significantly and independently associated with ALS. The components were composed of premorbid body weight, dietary fat intake, hypercholesterolemia and lipid modifying agents use, intake of anti-oxidants and leisure time activities respectively. Results of the differential co-exposition analysis of the exposome will be presented.

Conclusion: This study shows that leisure time physical activity, premorbid body weight, dietary fat intake and intake of lipid modifying agents are variables that do not convey the same risk signal in ALS, but have independent contributions to this risk. Since these factors all play a role in the cardiovascular profile or fitness of patients, this study supports the hypothesis that a benign cardiovascular risk profile or increased general fitness results in an increased risk profile for ALS.

OS 2.1: DEXPRAMIPEXOLE EFFECTS ON FUNCTIONAL DECLINE IN ALS PATIENTS IN A PHASE II STUDY: SUBGROUP ANALYSIS OF DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

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Objective: To explore treatment effects in different patient subgroups using post hoc analyses of data from Part 2 of a two-part, Phase II study of dexpramipexole.

Methods: ALS subjects (n=92) were re-randomized to dexpramipexole 300 mg/day or 50 mg/day for 24 weeks. Effects of dexpramipexole on the slope of the revised ALS Functional Rating Score (ALSFRS-R) and the Combined Assessment of Function and Survival (CAFS) were

evaluated in the following subgroups: riluzole use, gender, and site of symptom onset (bulbar vs limb). Other subgroups were formed using continuous variables dichotomized on median baseline values: age (<59 vs ≥59 y), ALSFRS-R (≤35 vs >35), slow vital capacity (<81% vs ≥81%), duration of symptoms (<18.7 vs ≥18.7 mo), diagnostic delay (<7.4 vs ≥7.4 mo), and progression rate (<0.7/mo vs ≥0.7/mo). For CAFS, a linear regression model was also applied to the data as continuous variables.
Results: Overall, there was a 21% reduction in ALSFRS-R decline favoring the 300-mg vs 50-mg group (p=0.177), and CAFS scores for the 300-mg group (52.4) were significantly higher vs the 50-mg group (41.1; P=0.046). These trends were replicated in all subgroups. With few exceptions, ALSFRS-R decline was less in the 300-mg vs 50-mg group across subgroups (p-values >0.07). With 1 exception, CAFS rankings were higher in the 300-mg vs 50 mg group across subgroups. CAFS rankings in the 300-mg group were significantly higher than in the 50-mg group among subjects with ALSFRS-R scores ≤35, symptom duration <18.7 months, or progression rate ≥0.7/month (p-values <0.03). Linear regression analysis was largely supportive; the 300-mg group had higher rankings vs the 50-mg dose group.
Conclusions: Results suggest that the observed benefit of 300 vs 50 mg dexpramipexole on functional decline and survival was generally consistent among subjects regardless of baseline demographic and clinical characteristics.

OS2.2: PROLONGED SURVIVAL AND MILDER IMPAIRMENT OF MOTOR FUNCTION IN THE SOD1 MOUSE MODEL DEVOID OF FGF-2 *N. Thau (Hannover Medical School, Department of Neurology)*

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by selective motoneuron loss in brain and spinal cord. Mutations in the superoxide dismutase (SOD) 1 gene account for 10-20% of familial ALS patients. The ALS-mouse model over-expressing a mutant human SOD1 (G93A) gene closely mimics human ALS disease. The cause for the selective death of motoneurons is still unclear, but among several pathomechanisms discussed, loss of neurotrophic factors is one possibility. Basic fibroblast growth factor 2 (FGF-2) plays a prominent role in the motor system. In order to evaluate a role of FGF-2 in ALS pathogenesis, double mouse mutants transgenic for the human SOD1 mutation and lacking the endogenous FGF-2 gene were generated. Both heterozygous and homozygous FGF-2 deficient mutant SOD1 mice showed a significant delay in disease onset and less impaired motor performance in comparison to mutant SOD1 mice with normal FGF-2 levels. Survival of the double mouse mutants was significantly prolonged for two weeks. Motoneuron numbers were significantly higher in the double mutants and astrocytosis was diminished at disease endstage. While one would initially have expected that FGF-2 deficiency deteriorates the phenotype of mutant SOD1 animals, our results revealed a protective effect of FGF-2 reduction. In search of the underlying mechanisms, we could show up-regulation of other neurotrophic factors with proven protective effects in the ALS mouse model, ciliary neurotrophic factor (CNTF) and glial derived neurotrophic factor (GDNF) in muscle and spinal cord tissue of double mutant animals.

OS2.3: EphA4 INHIBITION RESCUES THE MOTOR AXON PHENOTYPE IN A ZEBRAFISH MODEL FOR ALS AND SMA *Schoonaert Lies (Laboratory for Neurobiology, Experimental Neurology, KU Leuven, and Vesalius Research Center, VIB)*

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that specifically targets the motor neurons. Mutations in C9ORF72, superoxide dismutase 1 (SOD1) and TAR DNA-binding protein (TDP-43) are the most prevalent causes of familial ALS. A morpholino-based knockdown screen in a zebrafish model for the mutant SOD1-associated form of familial ALS was performed in order to identify factors affecting this phenotype. We identified receptor tyrosine kinase 2 (RTK2), a zebrafish orthologue of the human ephrin receptor A4 (EphA4), a member of the ephrin axon repellent system. Knockdown of RTK2 in fish expressing mutant SOD1 completely and dose-dependently rescued the motor axonopathy. To explore if exogenous intervention would yield similar results as genetic interference, we blocked the EphA4 receptor with a small compound, 2,5-dimethylpyrrolyl benzoic acid (here called C1). Addition of this compound to the Danieau water of mutant SOD1 overexpressing zebrafish completely rescued the motor axon phenotype. We also studied if the effect of EphA4 inhibition on mutant SOD1 overexpressing zebrafish would be similar when using another mutant gene. Overexpression of TDP-43 in zebrafish induces a similar axonopathy, which can be rescued by blocking the EphA4 receptor both genetically with a morpholino and pharmacologically with C1. Furthermore we investigated if blocking of the EphA4 receptor has a positive effect on another motor neuron disorder, spinomuscular atrophy (SMA). SMA is an inherited autosomal neurodegenerative disease caused by the homozygous deletion of Survival Motor Neuron-1 (SMN1). Knockdown of Smn in zebrafish embryos causes defects in motor axon outgrowth. Both EphA4 morpholino-knockdown as well as pharmacological inhibition completely rescued the phenotype. These observations suggest that the protective effect of blocking the axon repellent ephrin system may be generic to motor neuron and axonal degeneration.

OS2.4: A PHASE 2-3 TRIAL OF OLESOXIME IN SUBJECTS WITH AMYOTROPHIC LATERAL SCLEROSIS *Olesoxime for ALS Study Group (Centre SLA-IDF, Département de Neurologie, Hôpital de la Pitié-Salpêtrière, Paris)*

Objective: To assess the efficacy safety of olesoxime, a molecule with neuroprotective properties, in ALS patients treated with riluzole.
Methods: A double-blind, randomized, placebo controlled, multicenter trial of 18 months duration was conducted in 512 subjects with probable or definite ALS receiving 330 mg Olesoxime daily or matching placebo. The primary intention-to-treat analysis was 18 month survival. Secondary outcomes were rates of deterioration of ALS Functional Rating Scale–Respiratory (ALS FRS-R, focusing on the 9-month assessment), Slow Vital Capacity (SVC) and muscle strength. Blood levels, safety and tolerability of olesoxime were also assessed.
Results: At 18 months, 154 of the 512 ITT patients had died (79 of 253 placebo, 75 of 259 olesoxime). Estimated overall survival according to Kaplan-Meier analysis was 67.5% (95% CI, 61.0 to 73.1%) in the placebo group and 69.4% (95% CI 63.0 to 74.9%) in the olesoxime group; hence survival was not significantly different between treatment arms (p=0.71, stratified bulbar/spinal log-rank). Sensitivity analyses and other efficacy endpoints evaluated were also negative, with the exception of a small difference in ALS FRS-R global score at 9 months in favor of olesoxime but not after 18 months in either the bulbar or spinal sub-populations. Expected olesoxime plasma exposure was achieved in almost all evaluable olesoxime-treated patients, with high inter-patient variability. Overall survival was not

significantly different between the 3 pre-specified olesoxime exposure levels based on trough concentration. Treatment of this ALS population with combined riluzole and olesoxime did not raise any safety concerns.
Conclusions: Olesoxime, although well tolerated, did not show any beneficial effect in ALS patients treated with riluzole.

OS3.1: ELEVATED MITOCHONDRIAL CALCIUM EXTRUSION DISTRUBS ERMCC DYNAMICS IN G93A hSOD1 MOTOR NEURONS *J. Lautenschlaeger; T. Prell; J. Ruhmer; L. Weidemann; O.W. Witte; J. Grosskreutz (Hans-Berger Department of Neurology, Friedrich-Schiller-University Hospital Jena, Jena, Germany)*

Background: In ALS motor neurons display morphological abnormalities of mitochondria and the ER which are functionally coupled in the ER mitochondria calcium cycle (ERMCC). Decreased activity of mitochondrial complexes and the induction of the unfolded protein response (UPR) in sALS patients and mutant hSOD1 mice indicate functional disturbance which may also be present in TDP-43 and VAPB models. Intervention studies were able to show that modulation of UPR sensor proteins has a life extending effect in mutant hSOD1 mice.
Objectives: To determine mitochondrial ERMCC kinetics and functional state in the presence of disease causing G93A hSOD1.
Methods: Mixed motor neuron cultures were prepared from E13 ventral spinal cord of non-transgenic and G93A hSOD1 mice. Cytosolic calcium transients and mitochondrial membrane potential were monitored using fura-2 AM and rhod123.
Results: Upon application of Ru360, inhibiting the mitochondrial calcium uptake, motor neurons but not non-motor neurons failed to return kainate induced calcium levels to baseline. However, blocking of mitochondrial calcium export by CGP37157 caused severely increased calcium transients in non-transgenic motor neurons but not in G93A hSOD1 motor neurons. Furthermore, 100 sec kainate exposure caused a severe loss of mitochondrial membrane potential in non-transgenic compared to G93A hSOD1 motor neurons. A 12 h treatment with CGP37157 and CPA, reducing the calcium transport back to the ER showed a survival benefit for G93A hSOD1 motor neurons.
Discussion and Conclusions: These results shown, that mitochondria indeed play a prominent role in the calcium buffering of motor neurons, but mitochondrial calcium extrusion seems to be elevated in G93A hSOD1 motor neurons, especially mediated by the mitochondrial sodium/calcium exchanger. First survival analyses were able to show, that ERMCC stabilization may provide a new therapeutic principle in ALS.

OS3.2: PLCdelta1 KNOCKOUT PROLONGS SURVIVAL OF ALS MICE

Ms. Kim Staats (Department of Neurobiology and Vesalius Research Center, VIB, KU Leuven, Belgium)Staats, K. A., Van Helleputte, L., Lemmens, R., Thijs, V., Van Damme, P., Robberecht, W. & Van Den Bosch, L.

Phospholipase C hydrolyses phospholipids and releases diaglycerol and the second messenger inositol trisphosphate. The latter binds to its receptor and releases calcium from the endoplasmic reticulum. The delta 1 subtype of phospholipase C (PLCdelta1) is associated to a number of neurodegenerative diseases and is detected in inclusions in brains of Alzheimer's patients. This protein is also upregulated in neurons during excitotoxic stress and is necessary for nuclear shrinkage during this pathogenic process caused by the overstimulation of glutamate receptors. Excitotoxicity and disturbances in the calcium

metabolism play an important role in Amyotrophic Lateral Sclerosis (ALS). In this study, we investigated the potential effect of PLCdelta1 in ALS due to its described role in intracellular calcium homeostasis and excitotoxicity. No increased expression of PLCdelta1 is detected in ventral spinal cords of end stage ALS mice, despite the occurrence of excitotoxicity in this tissue. Analysis of gene expression in PLCdelta1 +/+, +/- and knockout spinal cords show comparable levels of motor neurons, astrocytes, inflammation, glutamate transporters and growth factors (as determined by quantitative PCR), allowing us to continue with behavioral studies and survival analysis of PLCdelta1 knockout in a mouse model of ALS (SOD1-G93A). Although PLCdelta1 knockout does not delay onset of SOD1-G93A mice, as measured by hanging wire, PLCdelta1 knockout significantly increases the survival of SOD1-G93A mice. Interestingly, gene expression analysis of PLCdelta1 in ALS patient lymphoblasts shows a trend of extended survival with lower levels of PLCdelta1. Our data suggests a moderate but beneficial role for diminished levels of PLCdelta1 in ALS, which may imply a role for decreased release of calcium from the endoplasmic reticulum in ALS.

OS3.3: MOLECULAR MECHANISMS OF GOLGI PATHOLOGY IN PROGRESSIVE MOTOR NEURONOPATHY *Sarah Bellouze, Michael K Schäfer, Catherine Rabouille, Georg Haase*
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Pathology of the Golgi apparatus represents one of the earliest features of degenerating motor neurons in amyotrophic lateral sclerosis (ALS) and related diseases but its molecular causes and mechanisms remain unclear. To investigate the potential role of microtubule defects and protein aggregates in ALS-linked Golgi pathology, we studied *pmn* mice with progressive motor neuronopathy which are mutated in the tubulin chaperone TBCE (Martin et al. Nat Genet 2002, Schäfer et al, J Neurosci 2007).
Here we demonstrate severe progressive Golgi fragmentation and atrophy in motor neurons of *pmn* mice by using immunofluorescence analyses, 3D organelle modeling and electron microscopy. Golgi cisternae were progressively transformed into a convolute of small vesicles. In parallel, the Golgi v- (vesicular) SNARE proteins GS15 and GS28 were drastically up-regulated while their corresponding t- (target-) SNARE protein Syntaxin-5 was present at normal levels. Golgi pathology in *pmn* motor neurons was completely rescued by transgenic wildtype TBCE but not mimicked by nerve axotomy indicating loss of TBCE function as its origin. The distinct effects of TBCE depletion, folding-deficient tubulin mutants and pharmacological microtubule disruption on Golgi structure in cultured motor neurons identified loss of microtubules rather than accumulation of misfolded tubulins as causative for Golgi pathology. Defective microtubule growth at Golgi membranes was shown to impede the traffic of Golgi-derived vesicles leading to their decreased docking and fusion at target membranes.
To our knowledge these data provide the first mechanistic explanation for Golgi pathology in motor neuron disease

OS3.4: ELEVATED PGC-1a ACTIVITY SUSTAINS MUSCLE FUNCTION THROUGHOUT DISEASE IN A MODEL OF FAMILIAL ALS *Philippe A Parone (Ludwig Institute for Cancer Research, University of California San Diego)*

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative disorder, characterized by selective loss of motor neurons and skeletal muscle degeneration. An early event is thought to be denervation-induced muscle atrophy

accompanied by alterations in mitochondrial activity and morphology within muscle. The transcriptional co-activator PGC-1a induces multiple effects on muscle, including increased mitochondrial mass and activity. We elevated PGC-1a levels using a transgenic approach in muscles of mice that develop fatal paralysis from an ALS-causing SOD1. This promoted an increase in mitochondrial mass and activity throughout disease course, together with the activation of other PGC1a-dependent pathways, and was accompanied by retention of muscle function, delayed muscle atrophy and significantly improved muscle endurance even at late disease stages. However, muscle denervation and motor neuron degeneration were unaffected and survival was not extended. This evidence demonstrates that mitochondrial dysfunction within muscle does not contribute to disease progression and that muscle is not a primary target of mutant SOD1 mediated toxicity, but that drugs increasing PGC-1a activity and mitochondrial activity in muscle represent an attractive therapy for maintaining muscle function in patients during progression of ALS.

OS3.5: UTILIZING A SOD1 ZEBRAFISH MODEL OF ALS TO DISSECT NEURONAL CIRCUITS INVOLVED IN ALS AND IN DRUG SCREENING *Dr. Tennore Ramesh (University of Sheffield)*

Introduction: Amyotrophic lateral sclerosis/ Motor Neurone Disease (ALS/MND) is a devastating neurodegenerative disease. Mutations in the SOD1 gene are one of the major causes of familial ALS. When, how and which neurons initiate the disease which starts focally and spreads through the nervous system remains unknown.
Objectives: Here we used a transgenic sod1 zebrafish model of ALS to track disease progression by utilizing a novel fluorescence reporter that labels stressed cells to address the longstanding questions in ALS, listed above. Cells respond to the presence of misfolded proteins by the induction of a heatshock stress response (HSR), which in our transgenic fish would produce red fluorescence. We hypothesized that misfolding of the sod1 protein in vulnerable cell groups would induce the HSR, marking cellular stress.
Methods: We will use our sod1 zebrafish model to track neuronal stress and use functional readouts to show that neuronal stress leads to neuronal dysfunction and damage.
Results: Mutant sod1 fish exhibit neuronal stress from as early as 24 hours post fertilization (hpf) indicating that the CNS is uniquely sensitive to the presence of mutant sod1. Interestingly, neuronal stress occurs earliest in the embryonic spinal inhibitory interneurons. The chronic loss of inhibition leads to stress in the motor neurons of symptomatic adults and we see reduction in the neuromuscular synapses formed from stressed motor neurons that innervate the muscles and lead to denervation. Riluzole, the only approved ALS drug can reduce this early neuronal stress. We have developed a highthroughput screen to identify neuroprotective compounds.
Conclusion: Our data provides compelling evidence that sod1 zebrafish model can be used to study early events in ALS pathogenesis and help in understanding neuronal circuits affected in ALS and in high throughput screening.

OS3.6: ALTERED EXPRESSION AND ACTIVITY OF A STEAROYL-CoA DESATURASE 1 IN AMYOTROPHIC LATERAL SCLEROSIS *F.SCHMITT, G.HUSSAIN, A.HENRIQUES, T.LEQUEU, JL.GONZALEZ DE AGUILAR, E.MARCHIONI, JP.LOEFFLER (INSERM U692. Université de Strasbourg.)*

ALS is a degenerative disease characterized by progressive loss of upper and lower motor neurons, muscle atrophy and paralysis. ALS neurodegeneration is associated with metabolic perturbations, including hypermetabolism and dyslipidemia, which points to abnormal

regulation of energy homeostasis. Our previous gene profiling studies on ALS muscle revealed down-regulation of stearoyl-CoA desaturase 1 (SCD1), which is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids. In this study, we showed that at 60 days of age, when mice do not present any clinical signs of motor impairment, SCD1 mRNA levels were lower in SOD1(G86R) than in wild-type muscle. At 90 days of age, when motor deficit usually starts, SCD1 expression was also lower in the transgenic mice, and this difference was more marked in animals with paralysed hind legs, at about 105 days of age. We also used gas chromatography to investigate the distribution of circulating and tissue fatty acids in relation to SCD1 activity. Major findings revealed that amounts of palmitoleic acid and oleic acid, products of the reaction catalyzed by SCD1, decreased in plasma of SOD1(G86R) mice from the pre-symptomatic stage. Additionally, a complete profile of the distribution of different fatty acids also showed differences in other lipidic species. In all, these findings indicate that SCD1 expression and activity is altered in ALS and hence provide evidence for a new molecular player associated with the neurodegenerative process.

OS4.1: EVIDENCE FOR SUBTYPES WITHIN THE COGNITIVE CONTINUUM: A POPULATION-BASED LONGITUDINAL STUDY *Elamin, M, Bede P, Byrne, S, Jordan N, Gallagher L, Lynch C, O\'Brien C, Wynne B, Pender N, Hardiman (Trinity college Intitute of Neuroscience)*

Background: Cognitive changes have been reported in up to 50% of patients with amyotrophic lateral sclerosis (ALS). It is unclear as to whether cognitive change is an integral part of ALS, or whether it represents a phenotypic marker of specific disease subtype(s) with distinct clinical characteristics.

Objective: To investigate whether cognitive status in ALS patients (1) changes with longitudinal follow up (2) predicts motor or cognitive deterioration with time.

Methods: This is a population-based, case-control, longitudinal study of cognitive function in incident ALS patients. Comprehensive clinical and neuropsychological assessments took place during home-visits that were undertaken at baseline, and then at six-monthly intervals until death or severe disability prohibited further participation. Age, sex, and education matched healthy controls also underwent repeated testing.

Results: 203 patients have been recruited to the longitudinal study. Mean age was 62.9 years, 60.1% were males, and 34.8% had bulbar-onset ALS. Median ALSFRS-R score at baseline was 38 (range 12-48). Second, third, and fourth assessments have been carried out in 100, 44, and 7 ALS patients respectively. Preliminary findings suggest that cognitive status at baseline is a significant predictor of rate of motor decline and risk of attrition due to death or severe physical disability. Cognitive stratification persisted on longitudinal analysis. Cognitively intact patients tended to remain cognitively intact(75-80%), with a minority developing language abnormalities. New-onset executive dysfunction was uncommon (3-5%), and was observed exclusively in patients with subtle findings at baseline. New-onset frontotemporal dementia (3/100 patients at six-month) was documented only in patients with frank executive and/or behavioural changes at baseline.

Conclusions: Longitudinal follow up of a population-based sample of ALS patients suggests that cognitive status may be indicative of non-overlapping disease subgroups that display with distinct rates motor and cognitive decline. Cognition is a useful clinical biomarker in ALS.

OS4.2: Quantitative analysis of corticospinal tract hyperintensity in ALS

Jez Fabes (University of Oxford, Nuffield Department of Clinical Neurosciences)

Background: Therapeutic development in ALS would benefit from diagnostic biomarkers to permit earlier administration of candidate drugs, and biomarkers that capture the phenotype heterogeneity. Diffusion tensor imaging (DTI) changes, including fractional anisotropy and mean diffusivity, have been consistently detected within the CST and corpus callosum (CC) of a range of ALS phenotypes, and appear faithful to post mortem neuropathological studies. However, a biomarker derived from a standard clinical MRI sequence in the routine work-up of patients with neurological symptoms would have particular appeal. T2-weighted corticospinal tract (CST) hyperintensity is frequently observed in amyotrophic lateral sclerosis (ALS), but regarded as insufficiently sensitive when reported visually.
Methods: Objective quantitative analysis of CST and CC intensity was performed on T2-weighted fluid-attenuated inversion recovery (FLAIR) images acquired at 3 Tesla from 49 patients (33 'classical' ALS, 10 'flail-arm' ALS, 6 primary lateral sclerosis (PLS)) and compared with 21 age-matched healthy controls. Voxel-wise permutation analysis was used to correlate FLAIR with DTI parameters.
Results: FLAIR intensity was significantly greater in the CST (p=0.001) and corpus callosum (p=0.003) of patients. FLAIR signal correlated spatially with DTI markers of degeneration in the CST and CC. Of the phenotype subgroups, the highest intensity was found in PLS and the lowest in flail-arm patients. Step-wise discrimination testing yielded a model composed of FLAIR intensity within the cerebral peduncles and the genu of the CC which combined with scoring of pathological reflexes, classified nearly 80% of patients into appropriate phenotypes. In a longitudinal analysis, the degree of increase in FLAIR intensity showed correlation with rates of disability and cognitive impairment progression.
Conclusions: Quantitative measurement of regional FLAIR intensity may have value in the diagnostic work-up of ALS patients. Clinical translation requires studies that include mimic disorders.

OS4.3: AMYOTROPHIC LATERAL SCLEROSIS SPREADS ALONG STRUCTURAL BRAIN CONNECTIONS *E Esther Verstraete,[1*] Martijn P. van den Heuvel,[2*] Jan H. Veldink,[1] Leonard H. van den Berg,[1] * authors contributed equally; Email: m.p.vandenheuvel@umcutrecht.nl [1] Department of Neurology, University Medical Centre Utrecht, Rudolf Magnus Institute of Neuroscience, The Netherlands [2] Department of Psychiatry, University Medical Centre Utrecht, Rudolf Magnus Institute of Neuroscience, The Netherlands*

Background: Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of upper and lower motor neurons. Patients usually experience focal symptoms at first with subsequent spread to other body regions. However, insights into how this fatal disease spreads and progressively affects the brain network - and the motor system in particular - are crucial for the development of effective treatment strategies. Here, we explored longitudinal effects of disease on reconstructed structural brain networks and questioned whether ALS progressively affects the brain network by involving an increasing number of connections over time or by progressive impairment of the same initially involved connections.
Methods: Twenty-five patients with ALS and 19 healthy control subjects were included. Patients were scanned twice with an average interval of 5.5 months. Whole brain Diffusion

Tensor Imaging (DTI) connectome imaging was performed, reconstructing structural connections of the brain's network in both patients and controls.
Results: The reconstructed brain networks in patients at different time points compared to controls demonstrated an expanding sub-network of affected brain connections over time (T=1 p=0.0030; T=2 p=0.0010), with a central role for primary motor regions (precentral gyrus and paracentral lobule). The affected brain connections did not show progressive involvement.
Conclusions: Our findings suggest that ALS is associated with a spatial spread of the disease, rather than progressively affecting a limited number of initially involved motor tracts. Our longitudinal findings provide MRI-based evidence of disease spread along structural connections of the brain network.

OS4.4: GREY MATTER CORRELATES OF CLINICAL VARIABLES IN AMYOTROPHIC LATREAL SCLEROSIS – A NEUROIMAGING STUDY *Dr. Peter Bede (Trinity College Dublin)*
Background: One of the major challenges of biomarker development in ALS is the significant disease heterogeneity. Site of onset, functional disability and extra-motor involvement are key components of heterogeneity in ALS. Ravits et al.(1) has proposed that ALS spreads focally in the CNS based on the somatotropic anatomy of motor neurons. Distribution of disease burden within the primary motor cortex has not been fully characterized in vivo to date, and regional motor cortex involvement has not been correlated with clinical disability.
Methods: We have conducted a large single centre neuroimaging study of a cohort of 33 ALS patients with no cognitive impairment and 44 healthy controls. A voxelwise generalized linear model was used to investigate the distribution of disease burden within the motor cortex in relation to clinical disability.
Results: The degree by which different body regions are affected in ALS corresponds to the degree of focal grey matter atrophy in the motor homunculus. Upper limb functional scores and bulbar scores correlate with cortical volume signals in the relevant parts of the motor strip. Additionally, regional grey matter differences between bulbar and limb onset patients also respect the functional architecture of the precentral gyrus. Thirdly, on a whole brain level, cortical ALS pathology extends beyond the motor cortex affecting frontal, occipital and temporal regions.
Conclusions: Focal grey matter atrophy within the primary motor cortex corresponds with functional disability in ALS. The findings support the existing concepts of cortical focality and motor phenotype heterogeneity in Amyotrophic Lateral Sclerosis. Reference: (1) - Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. Neurology. 2009;73(10):805-811

OS 5.1: EXPRESSION AND FUNCTION OF SYSTEM Xc- AND MICROGLIAL GLUTAMATE IN ALS MODELS *Mesci Pinar 1, Zaïdi Sakina 1, Barbeito Ana 1, Sato Hideyo 2, Mallat Michel 1, Boillee Severine 1 (1: INSERM UMRS-975, CNRS UMR-7225, UPMC, Paris, France. 2: Yamagata University, Yamagata, Japan)*
Although motor neurons are the cells degenerating in ALS, microglial cells participate to the progression of the disease but the specific microglial pathways implicated in motor neuron degeneration are still largely unknown. The aim of this study is to identify microglial-derived factors acting during the progressive phase of motor neuron degeneration. Considering the importance of excitotoxicity as a potential mechanism involved in ALS, we focused on excitotoxic glutamate released by microglial cells through system Xc- (specific subunit: xCT),

a cystine/glutamate antiporter, expressed by microglia and investigated if microglial glutamate and system Xc- are involved in motor neuron degeneration in ALS mice. We show that control or mutant hSOD1 expressing microglial cells, but not motor neurons, express xCT and to a higher level upon activation. Using primary microglial cells in culture, expressing or not xCT, we show that microglial cells release more glutamate (170%) when activated and this mainly through system Xc-, since xCT-/- microglial cells release 80% less glutamate and show no increased production upon activation. In addition, system Xc- is implicated in the production of other neurotoxic factors since xCT-/- microglial cells produce 70% less nitric oxide. These results implicate xCT as a potential mediator of microglial activation and function. In order to study the impact of system Xc- on ALS disease progression, slow progressing hSOD1G37R mice were mated to xCT-/- mice. As expected (since microglial cells are implicated in disease progression) age at disease onset was similar in hSOD1G37R:xCT-/- and hSOD1G37R:xCT+/+ mice. Following this cohort to end-stage will show if deleting xCT leads to a slower disease progression. In conclusion, system Xc- is implicated in microglial glutamate release and a decreased neurotoxic phenotype of microglial cells. Our study will unravel if system Xc- through microglial cells can affect motor neuron survival in ALS mice.

OS 5.2: ALTERED INTRACELLULAR CALCIUM SIGNALING CORRELATES WITH ASTROCYTE DEGENERATION IN ALS *Dr. Daniela Rossi (IRCCS Fondazione Salvatore Maugeri)*

Collective evidence indicates that motor neuron degeneration in amyotrophic lateral sclerosis is non-cell-autonomous and requires the interaction with the neighboring astrocytes. Recently, we reported that a subpopulation of spinal cord astrocytes degenerates in the microenvironment of motor neurons in the hSOD1G93A mouse model of ALS. Mechanistic studies in vitro identified a role for the excitatory amino acid glutamate in the gliodegenerative process via the activation of its inositol 1,4,5 triphosphate (IP3)-generating metabotropic receptor 5 (mGluR5). Since non-physiological formation of IP3 can prompt IP3 receptor (IP3R)-mediated Ca2+ release from the intracellular stores and trigger various forms of cell death, here we investigated the intracellular Ca2+ signalling that occurs downstream of mGluR5 in hSOD1G93A-expressing astrocytes. Contrary to wild-type cells, stimulation of mGluR5 causes aberrant and persistent elevations of intracellular Ca2+ concentrations in the absence of spontaneous oscillations. The interaction of IP3Rs with the anti-apoptotic protein Bcl-XL was previously described to prevent cell death by modulating intracellular Ca2+ signals. In mutant SOD1-expressing astrocytes, we found that the sole BH4 domain of Bcl-XL, fused to the protein transduction domain of the HIV-1 TAT protein (TAT-BH4), is sufficient to restore sustained Ca2+ oscillations and cell death resistance. Furthermore, chronic treatment of hSOD1G93A mice with the TAT-BH4 peptide reduces focal degeneration of astrocytes, slightly delays the onset of the disease, and improves both motor performance and animal life span.

OS 5.3: NEURO-MUSCULAR JUNCTIONS IN CO-CULTURES OF hiPSCs-DERIVED MOTONEURONS AND MYOTUBES *Marianne Stockmann (Institute for Anatomy & Cell Biology, Ulm University, Germany / Department of Neurology, Ulm Univer)*

Introduction: Neuro-muscular junctions (NMjs) define the connection of the axonal terminal of a motoneuron with the motor end plate, the highly-excitable region of the muscle fiber plasma membrane required for the initiation of action potentials. The molecular composition of the NMj is crucial for its function and maintenance whereas dysregulation of

endplate physiology is considered to be involved in denervation of the muscle cells and subsequent motoneuron degeneration. Thus, neuro-muscular contacts are an interesting target in investigating pathogenesis of motoneuron degenerative diseases. We reprogrammed human keratinocytes into human induced pluripotent stem cells (hiPSCs) and differentiated them into functional motoneurons. HiPSCs-derived motoneurons were further co-cultured with either primary or human myotubes in order to investigate the development of NMjs.
Objectives: We investigated the stage-dependent functional interplay of hiPSCs-derived motoneurons with primary rodent and human myotubes and finally, the generation of functional NMjs.
Methods: In the present study, we generated hiPSCs by reprogramming human keratinocytes followed by the differentiation into functional motoneurons. Additionally, hiPSCs-derived motoneurons were co-cultured with primary murine or human myotubes. Human myotubes were obtained from human muscle biopsies.
Results: Detection of spontaneous synaptic currents in electrophysiological measurements suggests functionality of in vitro differentiated motoneurons. Indeed, when co-cultured with primary or human myotubes, motoneurons induced accumulation of the AChR (acetylcholine receptor, visualized by α-bungarotoxin staining) on the membrane surface of the myotubes. Additionally, co-cultured myotubes showed increased frequency and more synchronous contractions compared to pure myotube cultures. On ultrastructural level, typical features of NMjs such as the infolding of the postsynaptic membrane could be detected. We investigated functionality of NMjs by inhibition of both the presynaptic transmitter release via botulinumtoxin A and the postsynaptic AChRs by curare application. In both cases, myotube contractions were significantly reduced only in neuro-muscular co-cultures, indicating the formation of functional NMJs.

OS6. DEBATE

OS 7.1: FUSopathy in cells and transgenic mice expressing an aggregation prone form of human protein *Vladimir L. Buchman (School of Biosciences, Cardiff University, United Kingdom)*

FUS and TDP-43 proteinopathies are characteristic features of certain forms of ALS and FTLD. These proteins share striking structural similarity, though the functional domains order in the FUS protein is inverse to that in TDP-43. Approximately 25 kDa C-terminal fragments of TDP-43 are commonly present in association with histopathological inclusions in the nervous system of patients with ALS-TDP. There is growing body of evidence that these truncated variants of TDP-43 contribute to the development of neurodegenerative changes. Here we addressed the question if a truncated variant of FUS with domain composition similar to that of truncated TDP-43 would trigger proteinopathy in cell culture models and in transgenic mice. In agreement with previously reported observations, in SH-SYS5 human neuroblastoma cells expression of FUS variants lacking functional nuclear localisation signal triggered formation of stress granules and accumulation of expressed proteins within them. However, the truncated variant of FUS formed different types of cytoplasmic structures in SH-SYS5 and other types of cultured cells. These structures displayed typical morphological and biochemical characteristics of intracellular inclusions consisted of aggregated proteins. Transgenic mice expressing the truncated variant of FUS under control of a Thy1 promoter in the majority of neurons developed neuronal pathology

at the age of 3 to 5 month that led to severe disability and death within 1-2 weeks after the onset of clinical signs. Multiple FUS-positive pathological cytoplasmic inclusions were observed in lower and upper motor neurons. The terminal stage of the disease was characterised by severe damage and loss of myelinated motor fibres in the ventral roots with sensory fibres in the dorsal roots much less affected. The loss of lower motor neurons was selective to certain discrete populations and coincided with the degree of neuroinflammation in the corresponding region. Thus, our FUS transgenic mouse model recapitulates many key features of ALS.

OS 7.2: ALS-ASSOICATED MUTANT VAPBP56S PERTURBS Ca2+ HOMEOSTASIS TO DISRUPT AXONAL TRANSPORT OF MITOCHONDRIA *Gábor M Mórotz (Kings College London, Institute of Psychiatry, Department of Neuroscience)*

A proline to serine substitution at position 56 in the gene encoding vesicle-associated membrane protein-associated protein B (VAPB; VAPBP56S) causes some dominantly inherited familial forms of motor neuron disease including amyotrophic lateral sclerosis (ALS) type-8. Here we show that expression of ALS mutant VAPBP56S but not wild-type VAPB in neurons selectively disrupts anterograde axonal transport of mitochondria. VAPBP56S-induced disruption of mitochondrial transport involved reductions in the frequency, velocity and persistence of anterograde mitochondrial movement. Anterograde axonal transport of mitochondria is mediated by the microtubule-based molecular motor kinesin-1. Attachment of kinesin-1 to mitochondria involves the outer mitochondrial membrane protein mitochondrial Rho GTPase-1 (Miro1) which acts as a sensor for cytosolic calcium levels ([Ca2+]c); elevated [Ca2+]c disrupts mitochondrial transport via an effect on Miro1. To gain insight into the mechanisms underlying the VAPBP56S effect on mitochondrial transport, we monitored [Ca2+]c levels in VAPBP56S expressing neurons. Expression of VAPBP56S but not VAPB increased resting [Ca2+]c and this was associated with a reduction in the amounts of tubulin but not kinesin-1 that were associated with Miro1. Moreover, expression of a Ca2+ insensitive mutant of Miro1 rescued defective mitochondrial axonal transport and restored the amounts of tubulin associated with the Miro1/kinesin-1 complex to normal in VAPBP56S expressing cells. Our results suggest that ALS mutant VAPBP56S perturbs anterograde mitochondrial axonal transport by disrupting Ca2+ homeostasis and affecting the Miro1/kinesin-1 interaction with tubulin.

OS 7.3: GENE EXPRESSION PROFILING OF LYMPHOBLASTOID CELLS FROM C9ORF72 RELATED AMYOTROPHIC LATERAL SCLEROSIS *Cooper-Knock J, Kirby J, Heath P, Rattray M, Shaw PJ.*

Introduction: Intronic hexanucleotide repeat expansions of C9ORF72 are found in approximately 10% of patients with amyotrophic lateral sclerosis (ALS) and represent the most common genetic variant. The function and dysfunction of C9ORF72 is poorly understood but it has been proposed that the expansion may mediate pathogenesis via a sequestration of RNA splicing factors. It is hypothesised that mechanisms of pathogenesis and novel therapeutic targets in C9ORF72-ALS will be discovered by gene expression profiling (GEP) in lymphoblastoid cell lines (LCL) from patients and controls.
Methods: GEP was carried out on RNA extracted from LCL of 10 ALS patients with the C9ORF72 expansion and 10 controls using Affymetrix HG-U133 Plus 2.0 GeneChips. Data was analysed with the Propagating Uncertainty in Microarray Analysis (PUMA) suite of tools. Aberrantly affected pathways were identified using the Database for Annotation,

Visualization and Integrated Discovery (DAVID). Differential expression of certain genes was validated by QRT-PCR. Results: 319 differentially expressed probe sets were identified in C9ORF72 ALS cases compared to controls. Significantly enriched pathways included 'RNA splicing' and 'chromatin modification'. Consistent with earlier studies C9ORF72 itself was down-regulated. Differentially expressed genes were successfully validated by QRT-PCR. Discussion: Identified down-regulation of splicing factors is consistent with sequestration which may mediate a broader disruption of RNA splicing. As other splicing associated genes were up-regulated, it is hypothesised that this represents compensation and a potential therapeutic target. A similar up-regulation of these genes has been discovered in FTLD-TDP. Certain results were comparable to gene expression in myotonic dystrophy 1 (DM1), another neuromuscular disease mediated by an intronic expansion. The extent to which disease mechanisms in C9ORF72-ALS and DM1 are similar remains to be determined.

OS7.4: C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION IN ITALIAN ALS PATIENTS

Dr. Nicola Ticozzi (Department of Neurology - IRCCS Istituto Auxologico Italiano)

Background: A hexanucleotide repeat expansion (RE) in C9ORF72 gene was recently reported as the main cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in the Northern European population, but its incidence is still unknown in Southern Europe.
Methods: We screened C9ORF72 RE in a large cohort of 259 familial (FALS), 1275 sporadic (SALS), and 862 healthy control individuals of Italian descent. The haplotype associated to C9ORF72 RE-carriers was evaluated and compared to the previously reported risk haplotype on chromosome 9p21.
Results: We found C9ORF72 RE in 23.9% (62/259) FALS, in 5.1% (66/1275) SALS and 0.2% (2/862) control individuals. Notably, two cases carried the C9ORF72 RE together with a mutation in two other ALS-associated genes. The phenotype of RE carriers was characterized by bulbar-onset, shorter survival, and association with cognitive and behavioural impairment. Extrapyramidal and cerebellar signs were also observed in families with RE. Genotype data for 10 single nucleotide polymorphisms surrounding C9ORF72 gene showed that 95% of RE carriers shared a restricted risk haplotype, detectable in only 27% of non-expanded ALS cases and in 28% of controls.
Conclusions: Mutations in C9ORF72 gene represent the most frequent cause of FALS and SALS also in populations of the Mediterranean area sharing a common founder with cohorts of North European ancestry. Although C9ORF72 RE segregates with disease, the identification of RE in controls and in patients with additional pathogenic mutations suggests that the penetrance and phenotypic expression of C9ORF72 RE may depend on additional genetic risk factors.

OS7.5: UNC13A INFLUENCES SURIVAL IN AN ITALIAN POPULATION-BASED SERIES

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Background: UNC13A gene, located on chromosome 19p13.3, is involved in the regulation of neurotransmitters release including glutamate. It has been recently shown that the common variant rs12608932 in gene UNC13A is associated with amyotrophic lateral sclerosis (ALS) susceptibility and may be an independent modifier of ALS survival in populations of North-European ancestry (Van Es et al, 2009; Diekstra et al, 2012).
Aim: To evaluate the effect of UNC13A on survival in a population-based series of ALS cases of Italian ancestry.
Methods: Two hundred sixty-one samples were genotyped on Infinium HumanHap550 beadchips (Illumina), and 236 samples were genotyped on Infinium HumanHap610-Quad beadchips (Illumina) according to the manufacturer's specifications; 535,468 SNPs were common across both platforms, including the rs12608932 single nucleotide polymorphism (SNP). Two hundred forty-seven ethnically matched controls were also genotyped. Survival was assessed with Kaplan-Meir curves and compared with the logrank test.
Results: The study included 497 apparently sporadic ALS patients (mean age of 61.6 years [SD 11]) resident in Piemonte, Italy. 134 patients (27%) had a bulbar onset. The minor allele frequency was similar in cases and controls (33% vs. 32%, p=0.91). However, we found a significant association with survival for both additive and recessive genetic models. With the latter model, patients carrying the CC (minor allele) genotype had a 12-months shorter survival than those carrying AA or AC genotypes (median survival time, CC 2.5 years [95% confidence interval, 1.5-3.4] vs. AA or AC 3.5 years [3.1-3.8]; p=0.017). This effect was independent from patients' age at onset, gender, site of symptom onset, phenotype and use of NIV and PEG in a stepwise forward Cox multivariable model (p=0.02; HR=1.42).Comment: In the Italian population, UNC13A has a strong independent modifier effect on ALS survival. This finding has implications relevant to both understanding ALS pathogenesis and in defining therapeutic interventions.

OS7.6: PGC-1 ALPHA AS A GENETIC MODIFIER IN EXPERIMENTAL ALS *B. Schwalenstöcker, K. Braunstein, K. Rona-Vörös, T. Wipp, D. Wiesner, A. Ludolph, P. Weydt (Department of Neurology, Ulm University, Germany)*

Background: Wasting and metabolic failure are important features of experimental and human ALS. The transcriptional co-activator PGC-1alpha is an important regulator of mitochondrial activity and biogenesis in many metabolically active tissues, including brain, muscle and fat. Recent clinical and experimental evidence from research into Huntington's disease and Parkinson's disease suggests that impaired function or activity of PGC-1alpha contributes to the pathogenesis of neurodegenerative disease spectrum disorders. Our data show that ALS transgenic mice in addition to their well-documented motor phenotype and weight loss show a variety of additional abnormalities suggesting involvement of PGC-1alpha mediated metabolic control.

Objectives: We aimed to investigate whether the lack of PGC-1alpha expression worsens the metabolic and motor phenotype of ALS transgenic mice and whether this has an effect on their survival.
Methods: We crossbred PGC-1a-/- and SOD1 (G93A, high copy) transgenic mice to generate PGC-1a-/-;SOD1(G93A) mice. As controls we used PGC-1a-/-;SOD1(wt), PGC-1a+/+;SOD1(G93A) and wild-type mice. Body weight, body temperature and blood glucose levels were measured in regular intervals starting at the age of 6 weeks. Additionally we performed evaluation of motor phenotype and string agility Results: Our data show that deletion of PGC-1alpha leads to an aggravation of the motor phenotype in ALS-mice as well as to a reduced life span in male SODG93A-mice. Dysregulation of body temperature is a common trait of SODG93A- and SODG93A-mice lacking PGC-1alpha expression, whereas fasting glucose levels of PGC1 alpha;SODG93A mice are reduced.
Discussion and Conclusions: Our results support a potential role of PGC-1alpha mediated metabolic regulation in the pathogenesis of ALS in SODG93A transgenic mice. Studies are ongoing to investigate whether activation of PGC-1alpha system has a beneficial effect in this disease model and could thus be exploited therapeutically. The work is funded by a grant from the Thierry-Latran-Foundation.

OS8.1: EXECUTIVE DYSFUNCTION IN ALS RELATES TO CHANGES IN WHITE MATTER INTEGRITY IN THE FRONTAL LOBES

Lewis Pettit, Bastin and Sharon Abrahams

Aim: This study aimed to determine whether cognitive impairments observed in ALS are underpinned by executive dysfunction or slowed processing speed, and to identify the cerebral loci associated with any observed impairments. Method: Cognitive functioning was investigated in 30 ALS patients and 30 age and IQ-matched controls using tasks designed to account for motor disability in ALS. Novel dual-task and processing speed paradigms were designed specifically to test the competing theories of executive functioning and speed. Background neuropsychological tests were also administered. In addition, diffusion tensor imaging (DTI) data was obtained, allowing measurement of white matter integrity through region of interest measures of fractional anisotropy and mean diffusivity of cerebral tracts. Results: ALS patients performed significantly worse than controls in the dual-task and spoken letter fluency test. However, the ALS patients performed comparably to controls on tests of processing speed. Patients' performance in the dual-task and spoken letter fluency tests was correlated with several white matter regions in the prefrontal cortex only including those adjacent to Broadman's area 10, Dorsolateral Prefrontal Cortex, as well as in the Genu and Frontal Association Fibres. Conclusion: The ALS group exhibited cognitive impairments indicative of executive dysfunction which were associated with extensive structural white matter changes in the frontal lobes.

OS8.2: COMPARISON OF THE KING'S AMYOTROPHIC LATERAL SCLEROSIS STAGING SYSTEM WITH THE REVISED AMYOTROPHIC LATERAL SCLEROSIS FUNCTIONAL RATING SCALE

R Balendra and Ammar Al-Chalabi

Background: A disease staging system is a set of milestones occurring in a specified order, representing progression through a disease. A staging system has been proposed for amyotrophic lateral sclerosis (ALS) consisting of four stages occurring at predictable proportions of the disease course: Stage 1 (first region involved i.e. symptom onset), Stage

2A (diagnosis), Stage 2B (second region involved), Stage 3 (third region involved), Stage 4A (need for gastrostomy) and Stage 4B (need for non-invasive ventilation).1
An established measure of functional decline in ALS is the revised ALS Functional Rating Scale (ALS-FRSr). While a functional scale is not a staging system, the ALS-FRSr nonetheless has a relationship with disease progression.
The two systems have some similarities. ALS-FRSr questions reflect regional involvement, corresponding to Stages 1-3 and need for gastrostomy or non-invasive ventilation. ALS-FRSr score < 5 indicates Stage 4B has been reached and a score < 7 indicates Stage 4 has been reached.
Objectives: To compare ALS Stage with ALS-FRSr in a cohort of ALS patients.
Methods: A database of ALS patients who had been recruited to a UK clinical trial between 2009 and 2011 was analysed. The ALS Stage and the concomitant ALS-FRSr score were recorded for each patient visit to the trial at baseline, week 12 and months 6, 9, 15 and 18. Median, range and interquartile range of ALS-FRSr was calculated for each ALS Stage.
Results: There were 217 ALS patients included in the study. 78.3% had limb onset and 21.7% had bulbar onset. 69.6% were male patients and 30.4% were female patients. ALS-FRSr scores were not normally distributed. The median ALS-FRSr scores with interquartile ranges at each stage were Stage 2A: 43.0 (41.0-45.0), Stage 2B: 38.0 (35.0-42.0), Stage 3: 34.0 (29.5-37.5), Stage 4A: 25.0 (19.25-31.75) and Stage 4B: 23.0 (18.00-29.25).
Discussion: The median ALS-FRSr score should decline as ALS progresses, and the spread of ALS-FRSr scores should widen since not all patients are equally functionally impaired, depending on the pattern and type of weakness. We observe both these effects, confirming that the ALS staging system used is a measure of disease progression.
Conclusions: The staging system measures progression through the disease while the ALS-FRSr reflects overall functional decline. The staging system is a useful additional measure which can be utilised in patient care and ALS research.
1. Roche J, Rojas-Garcia R, Scott K et al Brain 2012;135:847-852

OS8.3: SYSTEMIC DELIVERY OF ANGIOGENIN PROTEIN FOR THE TREATMENT OF ALS

Behan ÁT1, Coughlan K1, Cannon S1, Woods I1, Kieran D1, Prehn J1. (1Dept of Physiology, Royal College of Surgeons In Ireland, 123 St. Stephens Green, Dublin 2, Ireland)

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative condition where motoneurons in the spinal cord and brain stem die, resulting in paralysis and eventual death. Little is known about the causes of ALS, and there is no cure for this condition. A previous study has identified mutations in a gene encoding for angiogenin in ALS patients [Greenway et al., Nat. Genet., 2006]. Subsequent studies demonstrated that angiogenin protects cultured motoneurons against ALS-associated, stress-induced cell death [Sebastia et al., Cell Death Differ., 2009; Kieran et al., J Neurosci., 2008]. Furthermore, we have demonstrated that systemic angiogenin protein delivery significantly increased life-span and improved motor function in SOD1G93A mice [Kieran et al., J Neurosci., 2008]. These results also suggested that angiogenin protein delivery may be beneficial in treating patients with newly diagnosed ALS. Therefore, the aim of the current study is to focus on developing these findings into a therapeutic technology based on the delivery of systemic angiogenin protein for the treatment of ALS. We performed a dose-response investigation of the effect of systemic angiogenin protein delivery on angiogenin serum levels and angiogenin uptake and activity in the spinal cord of control and ALS mice (SOD1G93A). This was followed by the

establishment of a dose-response relationship of the effect of systemic angiogenin protein delivery on life-span and disease progression and motor function in a post-symptom onset treatment paradigm in ALS mice. Our pharmacokinetic studies demonstrated a marked difference in angiogenin uptake and elimination in the SOD1G93A mice compared to their wild-type counterparts. Additionally, angiogenin uptake was observed in the spinal cord of SOD1G93A mice following systemic intraperitoneal administration of angiogenin. Dose-response studies demonstrate an extension in lifespan, an increase in motor function and motoneuron survival in SOD1G93A mice following systemic angiogenin treatment from our post-symptom onset treatment paradigm. While endogenous Angiogenin was found to be enriched in motoneurons and to be released by motoneurons under stress conditions, endogenously released Angiogenin protein or exogenously delivered Angiogenin protein was preferably taken up by astoglia, suggesting that non-neuronal cells may be involved in the therapeutic effects of Angiogenin protein delivery. Together, our data provides a strong rationale for further exploring the beneficial effects of systemic angiogenin protein delivery for the treatment of ALS.

OS8.4: IDENTIFICATION OF NEURTOTROPHIC FACTORS FOR ALS-RELEVANT MOTOR SUBSETS BY A NOVEL FACS-BASED APPROACH *Dorothée Buttigieg, Arnaud Jacquier, Marc Barad, David Gentien, Pierre Delagrange, Georg Haase Institut des Neurosciences de la Timone, UMR 7289 – CNRS, Aix-Marseille University , 31 chemin Joseph Aiguier, F-13402 Marseille cedex 20, France, Tel +33 4 91 16 40 06, Fax +33 4 91 77 50 84, Email : georg.haase@univmed.fr*

Neurotrophic factors (NTF) represent promising therapeutic candidates for human ALS since they can enhance motor neuron survival during normal development and in rodent ALS models. Studies in knockout mice suggested however that subsets of motor neurons differ in their survival response to NTF. To identify NTF for ALS-relevant motor neuron subsets we here used a combination of novel flow cytometry, fluorescent-activated cell sorting (FACS) and transcriptomic techniques.

Motor neurons innervating limb, axial and abdominal muscles were identified in mouse spinal cord through the combinatorial expression of transcription factors ISL1/2, HB9, FOXP1, LHX1/2, LHX3 and OCT6 by using double-color flow cytometry. Limb motor neurons - which are vulnerable in ALS - as well as axial motor neurons - which are relatively resistant - were then isolated by FACS. Both motor neuron subsets were obtained with high yield and exquisite purity, seeded into 96 well plates, cultured in the presence of the neurotrophic factors BDNF, NT-3, LIF, CNTF, CT-1, GDNF, Neurturin, Artemin or IGF and monitored for survival. Dose-finding experiments revealed distinct survival responses of limb and axial motor neurons to HGF and CNTF. In line with these data, chip-based gene expression profiling, immunoblot and in situ hybridization analyses identified differential expression of the HGF receptor c-Met and the CNTF receptor Lifrbeta in subsets of limb and axial motor neurons.

In conclusion, this approach identifies the survival response of ALS-relevant motor neurons to various NTF and thereby provides a rationale for testing selected NTF alone or in combination in preclinical ALS trials.

Poster Session I
Friday 25th May, 18.00 – 19.30 (Chairs: Jesus Mora and Susanne Petri)

CLINICAL

P.01: SPINAL MRI ATROPHY STUDY IN AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL MUSCULAR ATROPHY

EL MENDILI, M-M; Cohen-Adad, J; Morizot-Koutlidis, R; Lenglet, T; Serge, R; Benali, H; Pradat, P-F (Laboratoire d\\\'imagerie fonctionnelle (LIF) U.678 INSERM / UMR-S UPMC)

It has been recently reported that a large proportion of patients with familial ALS and frontotemporal dementia are associated with a hexanucleotide repeat expansion in the first intron of c9orf72.

We describe a patient with a diagnosis of ALS-FTD with a psychiatric onset, carrying an expansion of c9orf72 gene. At the age of 50 he developed a depressive disorder characterized by somatization with gastroenteric and genitourinary symptoms, social isolation, lack of appetite and weight loss. Some months later, muscle weakness at the right hand occurred, followed by worsening of the mood disorder. The patient reffered to our ALS Centre. The collection of familial history revealed that the patient's father died after a 5 years history of ALS. Neither cognitive nor behavioural impairment was reported. Genetic analysis revealed a hexanucleotide expansion in the first intron of c9orf72 gene. MRI documented a marked hyperintensity along the corticospinal tract; MRI fiber-tracking revealed bilateral reduction of fractional anisotropy along the corticospinal tract. Brain PET-CT with 18FFDG presented reduced uptake of the radioactive tracer in the motor cortex bilaterally, in the fronto-mesial cortex bilaterally, between the anterior and the middle cingulate gyrus and in the postero-lateral occipital cortex bilaterally. The clinical and neuropsycological assessment was consistent with a diagnosis of FTD, associated to OCD, hallucinations and depressive mood disorder. Afterward the patient developed dysarthria, dysphagia, lower limbs weakness and hypotrophy and worsening of spasticity at upper and lower limbs. 13 months after the onset of the motor neuron disease, he is still alive, wheelchair-bound, with no evidence of respiratory failure, feeded with oral creamy diet. The association of ALS, FTD, depression, psychotic manifestations and OCD could set up a distinctive phenotype related to c9orf72 gene expansion. Nevertheless, this hypothesis needs to be confirmed by further observations.

P.02: REPORTING BIOMAKER – DEVELOPMENT IN ALS PATIENT TREATED WITH G-CSF MOBILIZED STEM CELLS

Prof. Ulrich Bogdahn (University of Regensburg - Department of Neurology)Jennifer Rösl, Andrei Khomenko, Dobri Baldaranov, Jochen Grassinger, Verena C. Haringer1, Katja Kollewe **, Renata Schreck, Susanne Petri **, Reinhard Dengler **, Albert Ludolph , Bernhard Kaiser, Michael Deppe***, Gerhard Schuierer#, Wilhelm Schulte-Mattler, Ulrich Bogdahn - Regensburg, San Diego, Ulm, Münster, Hannover*

Introduction: Treatment development in neurodegeneration is time consuming.Our study group selected prolonged open label autologous BM stem cell mobilization in human ALS-patients to validate reporting biomarkers for efficacy and safety.

Methods: 20 ALS patients were treated with s.c. rec-hu-G-CSF, in a standard 5/28 days or a 1/7 wk x 4 (= 1cycle) outpatient regimen: 5 to 10 µg/kg BW were given daily plus riluzole. Patients with a median 48 yrs (25-75y,13m - 7f,) were evaluated by ALS-FRS-R each 4 wks. Reporting biomarkers for disease modulation we (1) hypothenar muscle motor unit number

estimates (MUNE, Mc Comas) at 12 wks, (2) cranial MRI-DTI to delineate microstructural changes in motor cortex and pyramidal tracts at 12 wks,, and (3) BM function (Burst forming and colony forming units erythrocyte (BFU-E, CFU-E), granulocyte-macrophage (CFU-GM) and granulocyte-erythrocyte-monocyte-macrophage (CFU-GEMM). Safety included abdominal sonography, determination of BW, pulmonary function, clinical chemistry; stem-/precursor cells were monitored pre and post mobilization.
Results: Both application modes (5/28: 13,5 treatment cycles (4 - 25) / 1/7x4: 5,2 cycles (5 - 34) were safe, well tolerated, with no obvious difference in efficacy. Side effects were mild, tolerance excellent. Clinical outcome revealed some longer stabilisations, unrelated to age, dynamics, or mobilization. MUNE correlated to disease progression, however, showed increases in individual patients. DTI-/ FAI-values over time indicated minor efficacy. BM function was complex.
Discussion: Prolonged treatment with G-CSF is feasible and safe in ALS patients - study data are needed. MUNE. DTI potentially BM function are useful biomarkers.

P.03: SUSCEPTIBILITY MRI REVEALS ALTERED MAGNETIC BEHAVIOUR IN ALS-RELATED WHITE MATTER DAMAGE

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Background: As known from DTI and VBI analyses, MR-detectable white matter damage in ALS is primarily related to the corticospinal tract and corpus callosum. We discovered these regions to additionally exhibit altered signals in susceptibility weighted sequences and aim to further explore disturbance of magnetic behaviour as feature of neuron degeneration in ALS.
Methods: We obtained MR datasets from 30 ALS patients (mean ALSFRS-R 37.1, SD 6.1; mean age 62.7, SD 11.2; mean disease duration 23.5 months, SD 18.1) and 32 matched healthy volunteers containing a novel sequence called susceptibility weighted imaging SWI, as well as isolated phase and magnitude signal images, conventional T2* and T1 for anatomic reconstruction. All images were normalized to MNI space, masked for white matter and underwent voxel-wise ANCOVA of patients versus controls.
Results: In group comparison, all sequences show CST alterations, mainly above the internal capsule. Additionally, varying parts of the superior longitudinal fascicle and corpus callosum show altered magnetic behaviour between groups. ($p<0.01$, extent threshold 100 voxels).
Discussion: The location of alteration concurs with known areas of white matter degeneration in ALS. It is most likely that significant changes in magnetic behavior are due to myelin loss which is highly dependent on iron metabolism. Additionally, neurodegenerative diseases exhibit increased aluminum levels, for instance as in Alzheimer\'s plaques. Both, aluminum and iron, are known, when shifted in levels, to improve or worsen motor neuron disease. Another hypothesis suggests that, due to disturbed energy metabolism, chronically shifted levels of oxygen radicals would cause such findings. However, it is not clear yet, what it is exactly that causes altered signals in the sequences presented above. As they are capable of exhibiting known changes in ALS, they unveil new aspects of the disease and may contribute as a novel MRI biomarker.

P.04: DISTRIBUTION OF ADIPOSE TISSUE IN PATIENTS WITH ALS ASSESSED BY AUTOMATIC WHOLE BODY MRI ANALYSIS *H.-P. Müller1, E. Lindauer1, L.Dupuis1, H. Neumann2, J. Kassubek1, A.C. Ludolph1 (1Dept of Neurology, Univ. Ulm, Germany, 2Inst of Neural Information Processing, Univ. Ulm, Germany)*

Introduction: Neurodegenerative diseases are affecting body weight so that assessment of the body fat distribution in the course of the disease might be of interest as a surrogate marker. Body mass index and blood lipids have been reported to correlate with survival and functional status of ALS patients [1]. No information was available on regional distribution of adipose tissue in ALS patients despite important metabolic differences among fat depots. In this study, automated detection of fat tissue volumes from whole body MRI was used to detect changes in the body fat distribution between subcutaneous and visceral fat tissue volumes in ALS patients.

Methods: The whole body MRI data were acquired on a 1.5 T scanner by acquisition of 6 to 8 T1-weighted volumes, each consisting of 36 2-D slices. Sixty-two patients with ALS (age 60 ± 12 years, ALSFRS 36.3 ± 7.5, mean disease duration 22 months) and 62 age- and gender-matched controls were examined. By standardized image postprocessing protocol [2], subcutaneous and visceral fat was determined by the ATLAS (Automatic Tissue Labelling Analysis Software). Data were diffusion filtered prior to application of the fat determination algorithm ARTIS (Adapted Rendering for Tissue Intensity Segmentation) algorithm.

Results: The advantage of MRI-based fat volume determination was that the ROI could be restricted to parts of the body where major fat volume changes in the course of the disease were expected. Therefore, MRI-based fat volume determination was more sensitive for differentiation between subcutaneous and visceral fat. Total fat content of ALS patients was not changed as compared with controls. However, ALS patients displayed increased visceral fat content and an increased ratio of visceral to subcutaneous fat. Visceral fat content was not correlated with ALS-FRS-R, while subcutaneous fat in ALS patients correlated positively with ALS-FRS-R and leptin content. Multiple regression analysis showed that gender and ALS-FRS-R, but not site of onset, were significant predictors of total and subcutaneous fat.

Conclusion: This study showed the potential in determination of subcutaneous as well as visceral fat volume in selected body regions. Fat distribution is altered in ALS patients, with increased visceral fat as compared with healthy controls. Subcutaneous fat content correlated positively with functional status. These findings demonstrate that adipose tissue is affected in its topography in ALS and calls for further functional studies on this key metabolic tissue. References [1] Dorst J, Kühnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J Neurol. 2011; 258: 613-7. [2] Müller HP, Raudies F, Unrath A, Neumann H, Ludolph AC, Kassubek J. Quantification of human body fat tissue percentage by MRI. NMR Biomed 2011; 24: 17-24.

P.05: MONOCLONAL GAMMOPATHY IN THE FULL SPECTRUM OF MOTOR NEURON DISORDERS AND MULTIFOCAL MOTOR NEURON DISORDERS AND MULTIFOCAL MOTOR NEUROPATHY

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Objective: To determine the prevalence of monoclonal gammopathy in the full spectrum of motor neuron disorders and healthy controls.
Methods: Immuno-electropheresis and immunofixation techniques were used to determine the presence of monoclonal gammapathy in serum from 445 patients with amyotrophic lateral sclerosis (ALS), 60 patients with primary lateral sclerosis (PLS), 160 patients with progressive muscular atrophy (PMA), 88 patients with multifocal motor neuropathy (MMN) and 430 healthy controls. Demographic features, clinical characteristics, electrophysiological findings and survival data were recorded. Anti-GM1 IgM and IgG antibody titers were determined in sera from patients with MMN and PMA, and in sera from patients with ALS and PLS with monoclonal gammopathy. Logistic regression analysis was used to investigate associations of monoclonal gammopathy with motor neuron disorders.
Results: Prevalence of IgM monoclonal gammopathy was increased among patients with PMA (8%) (adjusted OR = 4.2; p =0.001) and MMN (7%) (adjusted OR = 5.8; p = 0.002) compared to controls (2%). Anti-GM1 IgM antibodies were present in sera from patients with PMA and MMN, but not in sera from other patient groups. The presence of IgM monoclonal gammopathy was associated with a relatively favorable disease course in patients with PMA.
Conclusion: IgM monoclonal gammopathy is associated with PMA and MMN, but not with ALS and PLS. These findings suggest that immunopathogenic mechanisms may underlie both PMA and MMN, and that a subgroup of patients with PMA may suffer from an immune-mediated motor neuron disease, which could offer new clues for therapy.

P.06: IMMUNOLOGICAL STATUS OF PATIENTS WITH ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of unknown cause, characterized by the involvement of both central and peripheral motor neuron. There is a reasonable suspicion that immune mechanisms are at least partly involved in the pathogenesis of ALS. The role of Borrelia burgdorferi in pathogenesis of ALS is unknown.
The aim of this study was to determine the frequency and significance of different immunological parameters in ALS patients.
Methods: Study comprised 112 patients diagnosed at the Neurogy Clinic, Clinical Center of Serbia. Following analyses were performed in all patients: 1) immunoserology (ANA, ANCA, antiparietal antibodies and circualting imunocomplexes); 2) serum and urin proteins electrophoresis with immunofixation (β2 microglobulin and paraprotein); 3) VDRL reaction and ELISA test for Borrelia burgdorferi (BB) in serum and cerebrospinal fluid.
Results: Pathological immunoserology was found in 20 (17.9%) patients and it was more frequent in females (p<0.05). Paraprotein and/or increased level of β2 microglobulin were identified 14 (12.5%) ALS patients. Positive test for BB and/or VDRL were found in 21 (18.7%) patients. At least one pathological result was recorded in 55 (49.1%) ALS patients.

Spinal onset of disease was more frequent than bulbar onset in patients with pathological results in comparison to those with completely normal immunological status ($p<0.01$). Conclusion: Positive immunoserology parameters are found in half of patients with ALS and they were especially common in females and patients with spinal onset of disease. Significance of this finding is still unknown and studies on a larger number of patients are needed.

P.07: ELECTRONIC PATIENT- REPORTED OUTCOME TO EVALUATE PHYSIOTHERAPY TREATMENT IN ALS

Robert Meyer; Christoph Münch; Andre Maier; Theresa Holm; Laura Steinfurth; Thomas Meyer (Department of Neurology, Charité-University Hospital, Campus Virchow-Klinikum, Augustenburger Platz)

Background: Current understanding of the role of therapeutic physical exercise in patients with amyotrophic lateral sclerosis (ALS) is limited. A few small studies have shown modest effects of moderate exercise in improving scores on functionality tests and decreasing disease symptoms. Furthermore, there is little evidence regarding treatment parameters, such as type, duration, frequency and intensity of physiotherapy. Further research will be critical for further progress physiotherapy treatment in ALS. Patient reported outcome (PRO) questionnaires in the treatment of complex chronic diseases are of increasing importance in clinical research, patient management and specialized care. Against this background, we investigate the effects of physiotherapy treatment in patients with ALS, using internet-based PRO measurement.
Methods: In a prospective, single-center clinical study, patients answer an electronic PRO questionnaire using a secure internet-based platform (ambulanzpartner.de). The Measure Yourself Medical Outcome Profile 2 (MYMOP2) is a validated, patient generated and problem specific questionnaire. It requires the patient to specify one or two symptoms which are most concerning and for which therapeutic physical exercise is provided. In addition, we use the Net Promoter Score (NPS), a customer loyalty metric, as a simple approach to patient feedback in a physiotherapy setting.
The study was started in March 2012. We seek to investigate 270 ALS patients in 24 months.
Discussion: Therapeutic physical exercise plays an important role in the current treatment of patients with ALS. However, there are no defined treatment guidelines and in clinical use physiotherapy treatment prescription considerations are relatively arbitrary. There is a lack of clinical trials examining therapeutic physical exercise in ALS patients. This study aims to investigate therapeutic physical exercise using an internet-based PRO questionnaire in a large ALS population.

P.08: ILLNESS BURDEN IN PATIENTS WITH ALS AND THEIR CAREGIVERS: A WEB-BASED SURVEY

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Background: There are currently numerous compounds in development for the treatment of amyotrophic lateral sclerosis (ALS). While it is hoped that these compounds will be successful in clinical trials and will have beneficial effect on survival and function, research is needed to quantify economic losses, caregiver burden, and quality of life impact associated with ALS at different stages of the disease. We sought to investigate lost productivity, caregiver burden, and quality of life among patients with ALS (PALS) and caregivers (CALS).

This survey is designed to explore relationships between PALS disease severity, functional impairment milestones and socioeconomic burden of CALS.
Methods: PALS and CALS who participate in PatientsLikeMe, an online data-sharing platform for people with serious illnesses, were invited to an online survey. Inclusion criteria for participation in the survey were: US residence, activity on PatientsLikeMe in the past 120 days, a self-reported diagnosis of ALS or identification as a CALS who serves as the main provider of help and care to a person with ALS; the CALS may be a spouse, parent, child, other relative, friend, or professional caregiver. Both PALS and CALS respondents completed information on demographics, the health and work performance questionnaire (HPQ), and the EuroQol EQ5D-3L. Additionally, PALS completed the ALSFRS-EX (Extension items) and the ALSAQ-5. CALS completed the Caregiver burden inventory (CBI).
Results: Data collection is ongoing and analyses will be completed in early 2012. Results will be presented.
Discussion: This project aims to describe the impact that ALS can have on PALS, CALS, and society more broadly, with a secondary goal of identifying specific inflection points in the disease that might trigger significant PALS and CALS burden.

P.09: SELF-ASSESSMENT OF THE DAILY FOOD INTAKE IN ALS VIA AN APPLICATION

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Background: Undesirable weight loss is common In ALS patients. For surviving in ALS the nutritional status is a well known prognostic factor. The early detection of alterations in food intake as well as changes in weight is essential for these patients. Commonly a nutrition consultation is performed after patients develop swallowing difficulties or suffer from weight loss. The nutritionist anamnestically determines a retrospective dietary protocol by conducting a standardized interview to evaluate the daily oral food intake for estimating the daily energy intake.
Methods: In a prospective study, patients record the oral food intake via an internet-based nutrition application by using a touchscreen tablet computer in their home care environment. Leaned on the established “quartered plate method” the web application shows different options of meals, portions and duration. Patients assess the portion and duration of every single meal according to a full plate compared to their normal food intake. Initially the nutritionist calculates the individual daily food intake and estimates individual mean portions. The self-assessment should be done at three predetermined days per week over a period of three months.
Results: We included 8 patients. Every patient was provided with a tablet computer with the installed application and a nutrition consultation was performed at the beginning. The intuitive user interface and the simple usability improve the compliance especially in patients with manual deficits.
Discussion: Web applications on tablet computers or on smart phones are well known by a wide range of Internet users. However, also patients, who never used such tools, quickly became familiar with this technique. To our knowledge we present the first web application for measuring the daily oral food and caloric intake in ALS. The study establishes the methodical feasibility and clinical tolerability of a web application for monitoring the daily food intake in ALS patients.

P.10: WEB-BASED SELF-ASSESSMENT OF DYSPNOEA IN ALS

André Maier (Charité - Universitätsmedizin Berlin)

Respiratory insufficiency is a prognosis determining syndrome and a crucial focus in palliative care of ALS patients. One of the main limitations of the established self-assessment ALS Functional Rating Scale-revised (ALSFRSr) is the lack of differentiation between neuromuscular hypoventilation and secretory pharyngeal and bronchial obstruction, which in itself represents an independent risk factor for ALS-associated dyspnoea. We present a study aimed at the obtainment of subjective assessments of different aspects of dyspnoea performed by patients themselves.
Methods: In a prospective, controlled and stratified study, patients conduct an online self-assessment of dyspnoea on the Internet portal www.ALShome.de. Inclusion criterion was a vital capacity of 60 % or less. To evaluate dyspnoea in ALS we employed the Cancer Dyspnoea Scale (CDS), which is an established tool in oncological indications. Simultaneous patients evaluate their respiratory effort on the established numerical Borg scale (0-10). The CDS comprises 12 items across three dimensions: subjective perception of sense of effort, anxiety and discomfort.
Results: We have included 30 patients in the study. To evaluate the validity of the scale an intersubscale correlation and a correlation of every single dimension with the total CDS was performed. There is a significant correlation of each dimension with the total CDS. Internal consistency was tested with Cronbach‘s alpha and test-retest reliability was analyzed with a correlation of each dimension and the total CDS after 7 days. The internal consistency and the test-retest reliability of the CDS are excellent.
Discussion: In contrast to the ALSFRSr, the CDS focuses on the multi-dimensional subjective experience of dyspnoea. We could show good test validity, a high reliability and a satisfactory contentual validity by high correlation with Borg’s scale and the ALSFRSr dyspnoea sub scale. The feasibility in the examined ALS population suggests a general application of the CDS in ALS.

P.11: TIME TO GENERALIZATION AS A PREDICATOR OF PROGNOSIS IN ALS

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Background: In Amyotrophic lateral Sclerosis (ALS) the outcome measures generally used as prognostic indicators are death or tracheotomy. The identification of early indicators of prognosis would allow physicians to program therapeutic interventions and to optimize the clinical trials’ design. The conversion from bulbar or spinal ALS to generalized ALS is a critical moment in the natural history of the disease and a possible clinical outcome. Objective: To evaluate if the time to conversion to generalized ALS may predict survival in ALS.
Methods: Patients were enrolled in the Centre of Motor Neuron Disease at the University of Bari, Italy (2004-2007). The study was terminated in February 2012. Neurological status was assessed at entry by revised ALS Functional Rating Scale (ALSFRS-r). “Time to conversion to generalized ALS (TTG)” was considered as the time of spreading from spinal or bulbar localization to both. Death or tracheotomy were used as outcome measures. Simple and multivariate logistic regression analysis were used to evaluated the correlation between TTG and 4-years mortality (adjusted for age, gender, site of disease onset, onset-diagnosis interval and ALSFRS-r at baseline).
Results: We enrolled 143 sporadic ALS, median age of 62 years (range: 29-85). Median onset-diagnosis interval was 13 months (range:1-127), median ALSFRS-r at baseline 38/48

(range:13-48). The median TTG was 16.4 months (range:2-137). Survival time was directly correlated to TTG (rs=0.6; p<0.0001). Logistic regression analyses revealed that TTG <1-year was associated with a 7-fold increased risk of 4-year mortality (OR=7.6; 95%CI:1.9-30.2, p=0.004), independently of possible confounders.
Discussion and Conclusions: In this clinical based prospective study we found that short time to generalization is a reliable predictor of worse prognosis in ALS patients.

P.12: WEIGHT LOSS IN ALS: REASONS, IMPACT ON QUALITY OF LIFE AND BENEFIT OF HIGH CALORIE SUPPLEMENTS *Körner S, Hendricks M, Kollewe K, Dengler R, Petri S*
Background: Weight loss (WL) is a frequent phenomenon in ALS. It occurs not only in association with dysphagia but also due to not yet fully understood disease-specific reasons. The aim of the present study was to investigate the extent of WL and its different reasons among ALS-patients. We further intended to analyse the impact of WL on mood or quality of life and the frequency of high-calorie supplement use and its benefit.
Methods: 121 ALS patients filled in three standardized questionnaires (Beck depression inventar (BDI), SF-36 questionnaire (quality of life), ALS-FRS_R) and were further interviewed about WL, possible reasons for WL and use/benefit of high calorie supplements.
Results: 56.2% of the patients suffered from WL. Thereof 38.2% had no dysphagia. In these patients eating habits and the prevalence of depression and fasciculations were equal to patients without WL. They did, however, significantly more often declare increased respiratory efforts. WL was associated with a significantly worse ALSFRS_R score and also with higher depression (BDI) and lower quality of life scores (SF36). Multiple regression analysis identified the ALSFRS_R score as confounding factor, showing that most of these differences were probably caused by the discrepancy in the ALSFRS_R. The difference in the SF36 subitem “Vitality” between patients with and without WL remained highly significant, which means that patients with WL feel more often exhausted, tired and spiritless. 33.8% of patients with WL consumed high calorie nutritional supplements and 70% of these reported subsequent weight stabilization or even weight gain.
Discussion and Conclusions: WL without dysphagia is frequent in ALS. One reason could be an increased respiratory effort and we assume hypermetabolism as another main reason. WL had a significant impact on quality of life. Substantial benefit can be obtained from high calorie supplements and they should therefore be given more frequently.

P.13: PHRENIC NERVE STUDIES PREDICT SURVIVAL IN AMYOTROPHIC LATERAL SCLEROSIS
Susana Pinto, Anabela Pinto, Mamede de Carvalho Translational and Clinical Physiology Unit, Institute of Molecular Medicine-Faculty of Medicine, University of Lisbon, Portugal.
Objective: Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease related to short survival due to respiratory failure. We aimed to test the predictive value for survival of the phrenic nerve motor in a large population of ALS patients.
Methods: We included 254 ALS patients followed in our tertiary center from 1997 and 2006 in whom phrenic nerve stimulation was performed and that fulfilled the study inclusion criteria. ALS was spinal-onset in 175 (group 1– G1) and bulbar-onset in 79 (group 2– G2). The following features were recorded at first visit: gender, age at symptom onset, onset region, diagnostic delay, forced vital capacity (FVC), ALS functional rating scale (ALS-FRS) including the respiratory subscore of the reviewed ALS-FRS, and mean amplitude of motor responses by phrenic nerve stimulation (PhrenAmpl). All patients were on riluzole.

Results: Survival analysis was evaluated by Kaplan-Meier log-rank test and multivariate Cox proportional hazards. Independent factors negatively affecting survival were bulbar-onset, elderly age at symptom onset, short diagnostic delay, FVC and small PhrenAmpl for the all population. Small PhrenAmpl and short diagnostic delay were also independent factors for G1 and G2, but age at onset and FVC were also independent predictors for G2.
Conclusions: Phrenic nerve stimulation is a powerful predictor of survival in ALS. It is non-volitional and easily standardized test which can be performed quickly in all patients. FVC and other respiratory tests depend on patient cooperation and are not reliable in patients with facial weakness. Significance: Phrenic nerve stimulation should be included in the routine assessment of ALS patients.

P.14: GAMMA-SYNUCLEIN PATHOLOGY IN ALS

Dr Owen Peters (Cardiff University)

We have previously demonstrated that mice expressing high pan-neuronal levels of γ-synuclein develop substantial motor deficits associated with the selective degeneration of motor neurons. Here we show that the protein may also play a role in human motor neuron disease pathogenesis. Immunohistochemical screening revealed a significant subset of amyotrophic lateral sclerosis (ALS) cases with a novel pathology characterized by the accumulation of γ-synuclein within the dorsolateral corticospinal tracts. The presence of γ-synuclein-positive profiles in the descending motor axon columns was associated with all stages of upper motor neuron atrophy. A subset of these profiles was found in association with phagocytic glial cells positive for HLA-DRα and Mac-2/Galectin-3. Sequential fractionation of proteins from the spinal cord tissues revealed detergent-insoluble γ-synuclein species specifically in the dorsolateral corticospinal tracts of ALS patients with γ-synuclein-positive profiles in this region. Our observations suggest that in the pathological aggregation of γ-synuclein might contribute to the pathogenesis of ALS.

P.15: IS THE FRONTAL ASSESSMENT BATTERY RELIABLE IN ALS PATIENTS?

Joost Raaphorst (Academic Medical Centre, Amsterdam, The Netherlands)

Introduction: The assessment of frontal functions in ALS patients is important because of the overlap with the behavioral variant of frontotemporal dementia (bvFTD). The Frontal Assessment Battery (FAB) has been suggested to be a feasible screening instrument in mild ALS. FAB-items rely on the ability to speak and move the (dominant) hand(s). It is unknown whether the FAB is reliable in moderate to severe ALS. We investigated the applicability and reliability of the FAB within a predominantly prevalent ALS cohort.
Methods: The FAB was administered to 93 ALS patients (85 ALS patients without a prior diagnosis of bvFTD and 8 ALS-bvFTD patients). Original scores and percentage of items that could be performed were recorded. Item-adjusted scores of the FAB were calculated as follows: Item adjusted score = original score * 100 / % of items performed. The ALS Functional Rating Scale Revised version (ALSFRS-R) was used to assess disease severity.
Results: Eight-seven patients (94%) had ALS symptoms of more than one year. The median disease duration was 36 months (0-328) and the mean ALSFRS-R was 31.4 (SD 8.8). Twenty patients (21.5%) were not able to perform one or more items of the FAB. The original FAB score correlated with the ALSFRS-R score ($r=0.30$; $p<0.01$) while the item-adjusted FAB score did not correlate with the ALSFRS-R score (-0.05, $p=0.61$). The item-adjusted FAB score was lower in ALS-bvFTD patients (66.7 (33.3-100) vs. 94.4 (38.9-100); $p < 0.01$) as compared

to ALS patients without bvFTD. The original FAB scores were not significantly lower in ALS-bvFTD patients (12 (6-18) vs. 16 (3-18).
Conclusion: One fifth of prevalent ALS patients cannot complete the FAB which limits the use of this instrument in longitudinal studies. The findings emphasize the importance of adjusting for motor impairment in cognitive and behavioral examinations of ALS patients.

P.16: LANGUAGE AND COGNITIVE IMPAIRMENTS IN BULBAR-ONSET AMYOTROPHIC LATERAL SCLEROSIS *Sarafov S, Raycheva M, Mehrabian S, Tournev I, Traykov L (University Hospital "Alexandrovska, Sofia, Department of Neurology)*

Reports show that about 50% of amyotrophic lateral sclerosis (ALS) patients have mild cognitive dysfunction, and there are a considerable number of case reports with agraphia, main on Japanese ALS patients. Nine Bulgarian ALS patients, similar in age (mean 63.9 +/- 6.5 years) and mean disease duration (2.9 +/- 6.1 years) were investigated, including tests of motor function and ALS progression; cognitive functions including general cognitive assessment (Mini Mental State Examination), memory (verbal learning), language assessment (Boston Diagnostic Aphasia Examination, Boston Naming Test). Each patient had developed dysarthria as an initial symptom; four patients cognitive impairment, more pronounced dysexecutive syndrome; five patients demonstrated progressive agraphia with no other cognitive domain affected (two of them had also behavior changes). Patients\' writing errors consisted of both syntactic and letter-writing mistakes. Different types of phonemic paragraphias (omissions, substitutions, displacements) were observed which progressed to neologistic ineligible writings. Such features have often been reported in Japanese patients with agraphia and ALS. The most frequent type of error was an omission of kana, the next most common was a missing grammatical subject. Writing errors were considered to be a specific deficit for some non-demented ALS patients.
Our results suggest that patients with bulbar-onset ALS may develop isolated agraphia as a single-domain cognitive impairment, preceding the clinical manifestation of aphasia or dementia. Language impairments, including progressive non-fluent aphasia and semantic dementia, which are also frontotemporal lobar degeneration subtypes have been linked to bulbar-onset ALS. The other patients could develop cognitive impairments, involving a dysexecutive syndrome. Increasingly, the non-motor cognitive manifestations of ALS can be considered to reflect a heterogeneous group of 'frontotemporal dysfunction syndromes' that include cognitive dysfunction, behavioural impairment and language deficits.

P.17: WHY DOES THE AGE AT ONSET OF AMYOTROPHIC LATERAL SCLEROSIS DIFFER BETWEEN COUNTRIES? *Susan Byrne, Iain Jordan, Marwa Elamin, Orla Hardiman (Trinity College Dublin, Ireland)*

Aims: To explain the differences seen between the mean ages at onset of ALS between countries. We propose that the mean age at onset of ALS is directly proportional to the life expectancy in that region.
Methods: In order to test this hypothesis all population based studies were reviewed and the mean age at onset of ALS was recorded. The mean age at onset was divided by the life expectancy in the country at the time of diagnosis in order to get a proportional age at onset. 95% confidence intervals were calculated and proportions were plotted to see if values fell within the range. A regression line was plotted to look for linear correlation. Residuals were tested for normality and variance was tested.

Results: Eight prospective population based studies and a further eight retrospective population based cohorts, including reports from Guam and the Kii peninsula, were identified. The proportional age at onset and diagnosis reported in prospective and retrospective population based studies was calculated and graphed (see figure 1). Regression analysis revealed that the mean age at onset correlated positively with the life expectancy for the region in which the study was undertaken(r= 0.91, p=0.01).
Discussion: As the mean age at onset of ALS maintains a constant relationship to life expectancy in a given population it could be argued that the factors that influence the life expectancy of a population also influence the onset of ALS. The increase in life span seen in recent times is attributable to environmental factors rather than genetic and evolutionary factors. But is healthy aging delaying the onset of ALS or is it allowing people who would have died of another condition before expressing the ALS phenotype to live long enough to develop it?

P.18: THE ALS REGISTER SWABIA: EPIDEMIOLOGY AND RISK FACTORS IN SOUTHERN GERMANY *A. Rosenbohm, D. Rothenbacher , G. Nagel, Albert C. Ludolph (Departments of Neurology and Medical Biometry and Epidemiology, Ulm University, Germany)*

ALS is usually regarded as a polygenic disease whose incidence may be determined by environmental factors. We established the first clinical-epidemiological register of ALS in Germany: in addition to the determination or estimation of incidence and prevalence in Swabia, extensive data are collected compatible with the questionnaire of the existing structures of the European ALS-MND group (EURALS). We create a first-time access to patients for the expansion of an already established and successfully operating biobank and gene bank. Besides the collection of patient-specific data on medical history, environment, lifestyle factors and the disease course, we perform a case-control study design with a 2-1 design (2 age-/gender and regionally matched controls over any case of disease). All patients of a geographically defined population group are captured due to collaboration with all neurological clinics in southern Germany. The ALS Register Swabia includes a target population of 8.6 million inhabitants and collects all motor neuron diseases that have occurred since 1.10.2008. All new cases since October 2010 are compared with healthy control subjects living in the same region and of the same sex and age. We collected about 400 retrospective cases during a two-year period 2008-2010 with a standardised incidence rate of 2,0. We find a high proportion of women >75 yrs which serves as an indicator for having reached completeness, since only good registries could reach this population group.
Conclusion: In the ALS register Swabia, we investigate potential risk factors such as physical activity, head injuries, and metabolic factors in a population-based case-control study. We hope to find out more about pathomechanisms. The combination of a clinical registry in a defined region with a population-based control arm allows to generate descriptive indicators and to calculate risk factors. We aim to elucidate the underlying pathophysiological mechanisms and to detect new therapeutic strategies.

P.19: MEDICATION USE, PAST MEDICAL HISTORY AND THE RISK OF ALS: A POPULATION-BASED CASE-CONTROL STUDY *Meinie Seelen, MD[1]; Perry TC van Doormaal, MD[1]; Margot HJ Roozekrans, MD[1]; Mark HB Huisman, MD[1]; Sonja W de Jong, MD[1]; H Jurgen Schelhaas, MD, PhD[2]; Anneke J van der Kooi, MD, PhD[3]; Marianne de Visser, MD, PhD[3]; Jan H Veldink, MD, PhD[1]; Leonard H van den Berg, MD, PhD[1]. (Utrecht, Holland)*

Objective: Sporadic amyotrophic lateral sclerosis (ALS) is believed to be caused by an interaction between genetic and environmental factors. Epidemiologic studies on medication use and antecedent medical diseases among ALS patients are sparse.
Methods: We studied the relation between medication use, past medical history and the risk of sporadic ALS, adjusted for confounders, in a prospective, population-based, case-control study in the Netherlands between 2006 and 2011. Data on medication and medical history were obtained by a structured questionnaire.
Results: A total of 679 patients and 2268 controls were included. The use of statins was associated with a decreased risk of ALS (adjusted OR 0.50, 95% CI 0.38-0.66). No association with other medications or antecedent medical diseases was found. However, use of drugs from the genito-urinary system, mainly including anti-testosteron agents, was associated with a shorter survival in ALS (HR 1.98, 95% CI 1.27-3.06).
Conclusion: The use of statins was negatively associated with the risk of ALS, consistent with a favourable lipid profile, which supports the hypothesis that a greater cardiovascular fitness might be a phenotypic expression of a genetic constitution of sporadic ALS and that an increased metabolic rate may play a role in the pathogenesis of ALS.

P.20: MAY HYPERTENSION HAVE PROTECTIVE EFFECTS IN ALS? RESULTS OF A POPULATION-BASED STUDY *Ilardi, Calvo, Moglia, Canosa, Cammarosano, PARALS, Mazzini, Mora, Chiò (CRESLA, Department of Neuroscience, University of Turin)*

Background: Factors related to cardiovascular risk have been assessed in ALS with uneven findings. A recent paper showed that a beneficial vascular risk profile is associated with an increased risk of ALS (Sutjeda et al, 2011).
Aim: To assess the effect of arterial hypertension in a population-based series of ALS patients. Methods. The study population were the 1260 ALS cases incident in Piemonte and Valle d'Aosta in the period 1995-2004. Patients were affected by definite, probable or probable laboratory-supported ALS. Arterial hypertension was indicated as systolic pressure ≥160 and diastolic pressure ≥100.
Result: A total of 272 patients (21.6%) were affected by hypertension at the time of onset of ALS. Hypertension was slightly more common among women (137 women [24.3%] vs. 135 men [19.9%], p=0.06). Patients with hypertension had a significantly lower age at onset of ALS than patients without hypertension in both genders (women, 68.1 [SD 8.3] years vs. 64.2 [11.9] years, p=0.0001; men, 67.8 [8.9] years vs. 63.7 [11.5] years, p=0.0001). No relationship between hypertension and site of onset (bulbar vs. spinal) was found. Patients with hypertension were more likely to have also diabetes mellitus (HR 2.9, 95% c.i. 1.8-4.6, p=0.0001) and frontemporal dementia (FTD) (HR, 1.8, 95% c.i. 1.1-2.9, p=0.01). Hypertension did not influence ALS outcome. Conclusion: We have found that a preceding arterial hypertension is associated to a delayed onset of ALS in population-based series. This finding is in line with the previous observation that a beneficial vascular risk profile is associated to an increased risk of ALS.

P.21: PREVALENCE OF IMMUNE-RELATED COMORBIDITY AMONG ALS PATIENTS IN A U.S. HEALTH INSURANCE CLAIMS DATABASE *JR Williams[1], DA Kerr[1], and W Farwell[1] [1]Biogen Idec, Cambridge, MA, USA*

Objective: Animal and human studies of amyotrophic lateral sclerosis (ALS) have reported immune system dysregulation in the central nervous system and periphery. Examples include neuroinflammation and changes in peripheral blood mononuclear cells such as T cells and monocytes. However, whether immune system dysregulation or potential immune cell dysfunction increases the risk of immune-related comorbidities in ALS is unclear. This study estimated the prevalence of immune-related comorbidities (autoimmune, hematologic, and infectious) among ALS patients within the i3 InVision Data Mart Multiplan database, a large U.S. health insurance claims database.

Methods: Subjects (n=1845) with ≥2 ALS medical claims (ICD-9 code 335.20) occurring between 2004 and 2011 were identified and age- and gender-matched to controls without a claim for any motor neuron diseases (n=3690). Comorbidity categories were defined using the Agency for Healthcare Research and Quality's Clinical Classification System. Prevalent comorbidity was defined as ≥1 medical claim with an ICD-9 code within a particular category. Twelve month prevalence and odds ratios (OR) were calculated for each comorbidity category.

Results: The prevalence of immune-related comorbidity in ALS subjects was as follows: "central nervous system infections" — 1.4% (OR=16.9), "immunity disorders" — 3.9% (OR=16.4), "systemic lupus erythematosus and connective tissue disorders" — 2.6% (OR=6.1), "other infections including parasitic" — 5.1% (OR=5.8), "cancer of lymphatic and hematopoietic tissue" — 2.7% (OR=3.3), "diseases of white blood cells" — 4.2% (OR=3.2), "bacterial infections" — 6.7% (OR=3.1), "mycoses" — 8.4% (OR=1.6), and "viral infections" — 6.3% (OR=1.2). All ORs were statistically significant ($p<0.05$).

Conclusions: ALS subjects in the database had an increased prevalence of immune-related comorbidity. Further studies should investigate the potential relationship between immune-related comorbidity and inflammatory or peripheral blood mononuclear cell biomarkers in patients with ALS.

Disclosure: This research was funded by Biogen Idec.

P.22: PREVALENCE OF CARDIOVASCULAR AND CEREBROVASCULAR RISK FACTORS AND COMORBIDITY AMONG ALS PATIENTS IN A U.S. HEALTH INSURANCE CLAIMS DATABASE *JR Williams1, DA Kerr1, and W Farwell1 (1Biogen Idec, Cambridge, MA, USA)*

Introduction: Data on the cardiovascular and cerebrovascular profile in ALS are limited. Some studies have demonstrated impairment of cardiac autonomic control in ALS. Data on cardiovascular and cerebrovascular disease risk factors are also limited in ALS. Dyslipidemia has been associated with both a decreased risk of ALS and increased survival in patients with ALS. This study estimated the prevalence of cardiovascular and cerebrovascular comorbidities and risk factors among ALS patients within a large U.S. health insurance claims database.

Methods: Subjects (n=1845) with ≥2 ALS medical claims (ICD-9 code 335.20) occurring between 2004 and 2011 were identified and age- and gender-matched to controls without a claim for any motor neuron diseases (n=3690). Cardiovascular comorbidity categories were defined using the Agency for Healthcare Research and Quality's Clinical Classification System. Prevalent comorbidity was defined as ≥1 medical claim with an ICD-9 code within a

particular category. Twelve month prevalence and odds ratios (OR) were calculated for each cardiovascular comorbidity category.
Results: The prevalence of cardiovascular and cerebrovascular risk factors in ALS subjects was as follows: “hypertension” (41.4%, OR=1.4), “lipid disorders” (28.2%, OR=0.8), “uncomplicated diabetes” (14.0%, OR=1; n.s.), “complicated diabetes” (6.1%, OR=1.2; n.s.). The prevalence of cardiovascular and cerebrovascular disease was as follows: “peripheral atherosclerosis” (5.6%, OR=1.6),” coronary atherosclerosis” (12.6%, OR=1.3), “acute cerebrovascular disease” (6.1%, OR=4.1),” transient cerebral ischemia” (1.8%, OR=2.8), “nonhypertensive congestive heart failure” (6.8%, OR=2.0), “cardiac dysrhythmias” (14.4%, OR=1.5), “cardiac arrest/ventricular fibrillation” (1.4%, OR=3.0), “nonspecific chest pain” (18.5%, OR=2.0), and “acute myocardial infarction” (2.7%, OR=2.6). ORs $p<0.05$ unless otherwise noted.
Conclusions: ALS patients in the database have an increased prevalence of cardiovascular and cerebrovascular disease. Hypertension was also more common in ALS patients, but diabetes and lipid disorders were not. Further studies should investigate the impact of cardiovascular and cerebrovascular disease on survival and quality of life in patients with ALS.

P23: EUROMOTOR *P.Berk Utrecht, Holland*

February 1st 2011 the European Seventh Framework project: European multidisciplinary ALS network identification to cure motor neuron degeneration, in short Euro-MOTOR, was started. The objective of Euro-MOTOR is to discover new causative and disease-modifying pathways to pave the way for novel therapies for ALS. Insights in the disease mechanisms of ALS can only be achieved in a large integrative effort at the European level. Major advances in the area of ALS genetics, proteomics, metabonomics, phenomics and exposomics have independently led to important progress in the field. The approach in the Euro-MOTOR project is to combine these elements. Euro-MOTOR aims to elucidate ALS disease mechanisms and common causative pathways by comprehensive systems biology approach to generate a robust and well-validated ALS model of disease. This in turn will provide validated targets and target combinations for the development of novel therapeutics. All of this will be achieved by pursuing the following 3 sub-objectives in 3 different pillars. These 3 pillars form the basis of the Euro-MOTOR project. Pillar I: To generate large scale quantitative data sets Pillar II: To integrate these data in a robust computational ALS model Pillar III: To validate this computational model and apply it for the development of novel therapies The central concept of Euro-MOTOR is that a robust and validated computational ALS model can only be generated when all its individual components are robust and validated. Therefore, each individual pillar is designed to deliver high-quantity, high-quality and validated data to the subsequent pillar.

POSTER SESSION II
Saturday, 26th May, 17.30-19.30 (Chairs: Albert Ludolph and Karen Morrison)

P.24: SCREENING FOR RARE VARIANTS IN THE CODING REGION OF ALS ASSOCIATED GENES AT 9p21.2 AND 19p13.3 *Max Koppers (University Medical Center Utrecht*)

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease that affects both upper and lower motor neurons. Genetic variants are thought to predispose to the disease. A large genome-wide association study identified two loci that increase susceptibility to ALS. These two loci on chromosome 9 and 19 include four genes, IFNK, MOBKL2b, C9ORF72, and UNC13a. A hexanucleotide repeat expansion in the non-coding region of C9ORF72 was recently identified as the cause of chromosome 9-linked ALS-FTD. Our aim was to determine whether the coding region of the associated genes harbour non-synonymous variants that play a role in ALS pathogenesis. We performed a mutation screening analysis in the coding regions of these four genes in DNA from 1019 sporadic ALS patients and 1103 controls from Dutch ancestry by re-sequencing. Pathogenic effect of variants was predicted using PolyPhen-2. A total of 19 coding rare variants were identified. Some of these were unique to ALS, but were detected only in a single patient. None of the genes showed significant enrichment of rare variants in the coding sequence. In addition, no enrichment for pathogenic variants was found. Rare variants in the coding region of UNC13a, IFNK, MOBKL2b, and C9ORF72 are unlikely a genetic cause for ALS.

P.25: ANALYSIS OF THE H63D POLYMORPHISM IN HFE AS SUSCEPTIBILITY FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS *Mr. Wouter van Rheenen (University Medical Center Utrecht)*

Background: Iron metabolism and oxidative stress are important fields of interest in the pathogenesis of amyotrophic lateral sclerosis (ALS). The H63D variant in the HFE (rs1799945) has frequently been associated with ALS and an online meta-analysis (www.alsgene.org) identified the H63D variant in HFE as an important susceptibility factor (OR = 1.72, 95% CI 1.20 – 2.48). These individual studies, however, exhibit small sample sizes and this association has never been studied in large genetic screens.
Objective: To study the association between the H63D variant in HFE and ALS in a large European cohort and to examine its effect on age at onset and survival. Methods: Included were 4,127 ALS patients and 4,949 control subjects from seven countries (the Netherlands, Germany, Belgium, Ireland, United Kingdom, Sweden and Switzerland). Genotyping of the H63D polymorphism was performed using Taqman assays.
Results: No effect of the H63D polymorphism on ALS susceptibility was observed (Odds Ratio: 1.02, 95% confidence interval: 0.93-1.11, p = 0.77). This variant did not affect age at onset or survival in ALS patients (p = 0.15 and p = 0.24 respectively).
Conclusion: The H63D variant in HFE is not associated with ALS. Publication bias might have affected previous studies and the role of HFE in ALS pathogenesis should therefore be reconsidered.

P.26: IDENTIFYING NOVEL RISK AND PROTECTIVE ALS VARIANTS THROUGH TARGETED RESEQUENCING *Kevin Kenna, Russell McLaughlin, Orla Hardiman, Dan Bradley (Trinity College Dublin)*

Introduction: Using molecular interaction, pathway and protein sequence data, we identified a set of candidate genes exhibiting functional relevance to known ALS genes. Through targeted resequencing we discovered multiple variants in a subset of these genes which may influence ALS risk. These include variants appearing to decrease as well as increase disease risk.

Methods: Candidate genes were selected through network and similarity analyses trained using 9 known ALS genes. Sequencing libraries were prepared as per the SureSelect protocol, using custom barcoded adapters. Libraries for 248 cases and 165 controls were resequenced using Illumina platforms. Reads were aligned using BWA. Quality control of alignments was performed using SAMtools, Picard and GATK. Variant calling, annotation and quality control was performed using GATK, SNPeff and custom scripts. Association testing (fisher exact test) was performed using PLINK/SEQ.

Results: 638 target genes revealed 3736 variants expected to affect protein structure. A subset were found to show nominal association. A proportion of these were over-expressed in controls, suggesting a protective effect. Variants were also found which occurred in multiple cases but neither sequenced controls nor individuals sequenced by the 1000 Genomes (n=1092) and NHLBI Exome Sequencing (n=5379) projects.

Discussion: The discovery of novel disease variants can provide new insight into disease mechanisms and provides biomarkers for clinical and research use. Despite a lack of translation into effective therapies, priority has been given to the discovery of variants driving disease in patients. Conversely protective variants preventing disease in healthy individuals receives comparatively little research interest, despite their potential to facilitate development of therapeutic strategies which don\'t require an understanding of the underlying pathological processes. Through targeted resequencing we have identified both potential risk and potential protective ALS variants, occurring across multiple genes with known functional relevance to previously discovered ALS genes.

P.27: IDENTIFY-BY-DESCENT MAPPING REVEALS GENOMIC LOCI THAT MAY HARBOUR MULTIPLE RARE ALS-CAUSING VARIANTS *Russell L McLaughlin, Kevin P Kenna, Dan G Bradley, Orla Hardiman (Trinity College Dublin)*

For most genes known or suspected to cause ALS, multiple mutations have been described. Therefore, for undiscovered genes, multiple mutations may play a role in the aetiology of the disease. The power of GWAS to detect such loci is reduced because different mutations are likely to arise within different haplotypes which may be tagged by opposite alleles of nearby SNPs. In this work, we attempted to address such issues by mapping regions of identity-by-descent (IBD) in Irish ALS cases and controls. Illumina 550k genotypes were phased using BEAGLE and regions of pairwise IBD were mapped in cases and controls using BEAGLE\\\'s fastIBD algorithm. Extent of within-cases IBD was compared to extent of within-controls IBD and significant deviations from expected values were considered to indicate possible locations of genes harbouring multiple mutations. This method identified three genomic loci with significantly higher IBD within cases than within controls. Deep resequencing of these regions may reveal disease-causing mutations that segregate with pairs of individuals with inferred relatedness.

P.28: HAS THE TIME NOW COME FOR A REVISION OF THE ALS GENE CLASSIFICATION SYSTEM? *Rubika Balendra, Ammar Al-Chalabi (Department of Clinical Neuroscience, King\'s College London)*

Aim: The aim was to design a rational Amyotrophic Lateral Sclerosis (ALS) genetic classification.

Background: The past twenty years have seen great advances in identifying genes causing ALS and a numbered gene classification system currently exists consisting of fourteen genes. However this classification system does not accurately reflect the clinical syndrome of ALS and there are several anomalies. For example, genes for very slowly progressive motor syndromes, for juvenile onset, or for pure upper motor neuron syndromes are over-represented and listed as ALS genes. On the other hand, genes known to be responsible for a significant proportion of ALS are listed as ALS-FTD (Frontotemporal Dementia) genes. We therefore propose an overhaul of the existing ALS genetic classification system.

Methods: Clinical syndromes of genetic ALS were compared with criteria for classical adult-onset ALS. Genes known to cause ALS as a phenotype were reviewed for classification as ALS genes. Results: Of the fourteen current ALS genetic syndromes, nine are consistent with classical adult-onset ALS. A further two syndromes currently not classified as genetic ALS could be included in a new system.

Conclusion: The current classification system is derived from genetic studies which are driven by historically available genetic techniques rather than the clinical syndromes seen in ALS clinics. A rational redesign would benefit clinicians, patients and researchers.

P.29: ALS-CAUSING MUTANT TDP-43 VARIANTS AND THEIR EFFECT ON PROTEIN DEGRADATION PATHWAYS *H.Wolf(1), A.Besemer(1), M.Stark(1), I.Drechsler(1), H.Witan(1), JP.Julien(2), C.Behl(1), A.Clement(1) (1)Institute for Pathobiochemistry,University Medical Center,Johannes Gutenberg University Mainz,Ger)*

A pathological hallmark of many neurodegenerative diseases like ALS is the appearance of ubiquitin-positive protein aggregates in affected tissues in the course of disease. TDP-43 has been identified as the major pathological protein of aggregates in ALS and FTLD-U. TDP-43 is a DNA/RNA-binding protein with various fundamental functions that is well conserved among species and its protein level is self-regulated by a negative feedback-loop. Mutations in the gene coding for TDP-43 (TARDBP) have been genetically linked to 3-6% of all ALS-cases, suggesting that missense mutations in TDP-43 can cause neurodegeneration. The accumulation of aggregation-prone molecules is at least in part mediated by an insufficient activity of the protein degradation machineries, in particular macroautophagy (hereafter referred to as autophagy), possibly caused by these proteins themselves. Autophagy is responsible for the clearance of most long-lived proteins, dysfunctional organelles and protein aggregates, and a low level of constitutive autophagy is important for the normal turnover of proteins. In vitro studies showed that TDP-43 is mainly degraded by the autophagic pathway. On the other hand, a knock-down of TDP-43 resulted in the destabilization of Atg7 mRNA and consequently in the impairment of autophagy. These data clearly indicate that mutant TDP-43 might interfere with protein degradation pathways, in particular autophagy. In this study, we investigated the impact of two ALS-causing mutant variants of TDP-43, TDP-43G290A and TDP-43Q331K, on autophagy. We generated neuroblastoma N2A cell lines that stably express mutant TDP-43 variants and wild type TDP-43 linked to EGFP. The expression of mutant TDP-43 resulted in an altered cellular morphology and displayed toxicity under normal conditions. As we could show that mutant

variants TDP-43 impair basal autophagy, we propose that mutant TDP-43 acquires one or more toxic property(ies) when misplaced in the cytosol. (2) Research Centre of CHUQ/Department of Psychiatry and Neurosciences, Laval University Québec, Canada

P.30: P2RX7 POLYMORPHISMS AND SUSCEPTIBILITY TO ALS IN AN ITALIAN POPULATION: PRELIMINARY RESULTS *Michele Benigni[1], Claudia Ricci[1], Stefania Casali[1], Giannini Fabio[1], Cinzia Volonté[2], Stefania Battistini[11]Department of Neurological, Neurosurgical, and Behavioral Sciences, University of Siena, Siena, Italy[2]Cellular Biology and Neurobiology Institute, CNR Fondazione Santa Lucia, Rome, Italy*

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease characterised by neurodegeneration of motor neurons. Approximately 90% of the ALS cases are sporadic (SALS). To date, the etiology of SALS is undisclosed and little is known about the factors contributing to its development. The collective evidence is that ALS is a non-cell autonomous disease and motor neuron damage depends on the active participation of non-neuronal cells such as microglia and astrocytes. Extracellular release of ATP through P2 receptors constitutes a well known neuron-to-microglia alarm signal and purinergic mechanisms were suggested to play a novel role in ALS pathogenesis. Among P2 receptors, the P2X7 subtype, coded by the P2RX7 gene, is mainly expressed in CNS by microglia, and exerts proinflammatory effects. Activation of P2X7 receptor is often observed during neuroinflammation and neurodegeneration. Two substitutions in P2X7 protein, Q460R and E496A, have been suggested to be associated with several diseases. To date, no studies have been performed about the involvement of P2RX7 gene in ALS. In this work, we have evaluated the role of both Q460R and E496A gene variants as susceptibility/modifying factor in ALS patients. 150 Italian ALS patients and 255 controls have been studied for Q460R and E496A substitutions by DHPLC and RFLP. Statistical analysis of polymorphism frequencies has shown no significant differences between patients and controls, at genotype, allele and haplotype level. Within the patients group, genotype and haplotype analysis has not revealed significant correlation with gender, age, site of onset. However, survival analysis has evidenced a significant association between the presence of Ala in the E496A variant and an increase in disease duration. The Glu-496 to Ala substitution is known to lead to loss of P2X7 receptor function. Our preliminary results, that need further validation, would sustain an attenuation of microglia activation and a consequent neuroprotective effect.

P.31: SYSTEMIC DEPLETION OF SCD1 PROMOTES ACCELERATED MOTOR FUNCTION RECOVERY FOLLOWING NERVE INJURY *G Hussain, F Schmitt, F Rene, A Henriques, J-L Gonzalez De Aguilar, J-P Loeffler (Inserm, Umrs692, Université de Strasbourg, Strasbourg, France)*

The dismantlement of neuromuscular junctions (NMJs) is typically observed in degenerative conditions of the lower motor neurons, such as in ALS. Neurodegeneration in this disease is associated with metabolic perturbations, including hypermetabolism and dyslipidemia, which points to abnormal regulation of energy homeostasis. Our previous gene profiling studies on ALS muscle revealed down-regulation of delta-9 desaturase (SCD1), which is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids. In addition, SCD1 deficiency is known to stimulate muscle beta-oxidation. Here we investigated whether a decrease in SCD1 expression was involved in the maintenance and function of NMJs. The genetic ablation of SCD1 was not detrimental per se to the neuromuscular function. On the contrary, muscles in SCD1 knockout mice showed a metabolic shift toward a more oxidative

phenotype (increased amounts of SDH positive fibers and expression of PGC1-alfa), and an enhancement of synaptic gene expression (acetylcholine receptor subunits and MuSK). Lacking SCD activity, by genetic (SCD1-KO) or phamacological (SCD enzymatic inhibitor) means, accelerated the recovery of motor function after inducing sciatic nerve crush, as determined by measures of muscle grip strength and electromyography. Altogether, these findings provide evidence for a new role of SCD1 in coordinating NMJ remodeling, and in modulating the regenerative capacity of the neuromuscular axis.

P.32: GENETICS OF ALS IN ITALY: RESULTS OF A POPULATION-BASED STUDY

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Background: According to a recent systematic review, 5% of ALS patients have a positive family history for the disease (FALS) (Byrne et al, 2011). Of these, 20 to 30% carry mutations of SOD1, TARDBP, FUS, and OPTN, and another 40% carry a mutation of the recently described C9ORF72 gene. About 1 to 5% of apparently sporadic cases (SALS) also carry a mutation of one of these genes. No population-based studies have systematically studied the frequency of mutations of these genes in a well defined population.
Aim: To assess mutations of SOD1, TARDBP, FUS, OPTN and C9ORF72 in ALS in an epidemiological setting.
Methods: We report the data about the frequency of positive family history and genetic mutations in all ALS cases diagnosed from January 2007 to June 2011 in Piemonte, Italy.
Results: Out of a total of 474 cases, 45 (9.5%) had a positive family history for ALS or FTD. Forty-nine patients (10.3%) carried a mutation of one of the ALS-related genes, including 30 FALS (66.7% of FALS) and 19 apparently SALS (4.4% of SALS). A total of 31 patients (6.5% of all ALS cases) carried a hexanucleotide expansion in the first intron of the C9ORF72 gene, 9 (1.9%) had missense mutations of SOD1, 7 (1.5%) missense mutations of TARDBP, 1 a missense mutation of FUS and 1 a missense mutation of OPTN.
Discussion: In this epidemiologic study, the frequency of FALS was higher than that reported in the systematic review, probably because of the intensive assessment of patients' pedigrees. Globally, 10% of patients carried one genetic mutation, with C9ORF72 representing more than half of the mutations. Of note, 4% of apparently SALS carried a mutation of one of the ALS-related genes. These findings may modify the attitude of neurologists toward the execution of genetic testing in ALS patients.

P.33: CGG-repeat expansion in FMR1 is not associated with amyotrophic lateral sclerosis

Ewout J N Groen[a,b], Wouter van Rheenen[a], Max Koppers[a,b], Perry T C van Doormaal[a], Lotte Vlam[a], Frank P Diekstra[a], Dennis Dooijes[c], R Jeroen Pasterkamp[b], Leonard H van den Berg[a], Jan H Veldink[a]

Recently, repeat expansions in several genes have been shown to cause or be associated with amyotrophic lateral sclerosis (ALS). It has been demonstrated that an intronic hexanucleotide repeat expansion in C9ORF72 is a major cause of both familial (~40%) and sporadic (~5%) ALS, as well as frontotemporal dementia (FTD). In addition, CAG-repeat expansion in exon 1 of ATXN2, otherwise known to cause spinocerebellar ataxia type 2, has been identified as a major risk factor for sporadic ALS. Intermediate repeat expansions in the fragile X mental retardation 1 (FMR1) gene (55 to 200 repeats) are known to cause fragile X-associated premature ovarian insufficiency ((FX)POI, female carriers) or fragile X-

associated tremor/ataxia syndrome (FXTAS, male carriers) by CGG-mediated RNA toxicity. The present investigation involves screening FMR1 repeat length in 742 sporadic ALS patients and 792 matched controls. Our conclusion is that FMR1 repeat expansions are not associated with ALS.

P.34: ASSOICATION OF FUS WITH THE CYTOSKELETAL PROTEINS TBCB AND DCTN1
Stefan Putz (University Hospital Ulm)
The ALS related protein FUS (Fused in Sarcoma) is a multifunctional RNA binding protein which is involved in multiple steps of RNA processing such as splicing, translation and transport. Most of the ALS causing FUS mutations affect the nuclear localization signal and lead to a mislocalization of the protein into the cytoplasm. It is unknown whether this mislocalization causes a loss of function in the nucleus or a toxic gain of function in the cytoplasm. In order to identify interaction partners of FUS which could be affected by this mislocalization, we performed a yeast two hybrid screen with FUS as a bait. Amongst other proteins we found the cytoskeletal associated protein TBCB (Tubulin-folding cofactor B) as an interaction partner of FUS. TBCB is a protein which is involved in microtubule dynamics and plays a role in neuronal growth cones. Interestingly TBCB also interacts with dynactin-1 (DCTN1) which is a regulator of intracellular transport along microtubules. Same as FUS, mutations in the DCTN1 gene have been linked to ALS. Cotransfection experiments with cell lines and primary neurons revealed that FUS not only colocalizes with TBCB but also with DCTN1. We observed that the overexpression of DCTN1 recruits cytoplasmic FUS into the filamentous structures of the cytoskeleton. Thereby our mutated forms of FUS with a high cytoplasmic distribution show the highest degree of colocalization with DCTN1. Our subsequent pulldown experiments and microtubule binding assays further confirm a strong association of FUS with the microtubule cytoskeleton and hint to an interaction between FUS and DCTN1. Based on these first findings we plan to further elucidate the interaction network between FUS, TBCB and DCTN1. Furthermore we are interested in the functional relevance of these interactions and the possible impact of ALS related DCTN1 and FUS mutations.

P.35: AMYOTROPHIC LATERAL SCLEROSIS CARRYING EXPANSION OF C9ORF72 WITH OBSESSIVE-COMPULSIVE DISORDER AT ONSET *Moglia C, Calvo A, Canosa A, Ilardi A, Bersano E, Manera U, Restagno G, Chio' A ALS center, Department of Neuroscience, University of Torino, Italy*
It has been recently reported that a large proportion of patients with familial ALS and frontotemporal dementia are associated with a hexanucleotide repeat expansion in the first intron of c9orf72.
We describe a patient with a diagnosis of ALS-FTD with a psychiatric onset, carrying an expansion of c9orf72 gene. At the age of 50 he developed a depressive disorder characterized by somatization with gastroenteric and genitourinary symptoms, social isolation, lack of appetite and weight loss. Some months later, muscle weakness at the right hand occurred, followed by worsening of the mood disorder. The patient reffered to our ALS Centre. The collection of familial history revealed that the patient's father died after a 5 years history of ALS. Neither cognitive nor behavioural impairment was reported. Genetic analysis revealed a hexanucleotide expansion in the first intron of c9orf72 gene. MRI documented a marked hyperintensity along the corticospinal tract; MRI fiber-tracking revealed bilateral reduction of fractional anisotropy along the corticospinal tract. Brain PET-

CT with 18FFDG presented reduced uptake of the radioactive tracer in the motor cortex bilaterally, in the fronto-mesial cortex bilaterally, between the anterior and the middle cingulate gyrus and in the postero-lateral occipital cortex bilaterally. The clinical and neuropsycological assessment was consistent with a diagnosis of FTD, associated to OCD, hallucinations and depressive mood disorder. Afterward the patient developed dysarthria, dysphagia, lower limbs weakness and hypotrophy and worsening of spasticity at upper and lower limbs. 13 months after the onset of the motor neuron disease, he is still alive, wheelchair-bound, with no evidence of respiratory failure, feeded with oral creamy diet. The association of ALS, FTD, depression, psychotic manifestations and OCD could set up a distinctive phenotype related to c9orf72 gene expansion. Nevertheless, this hypothesis needs to be confirmed by further observations.

P.36: FASTER DISEASE PROGRESSION IN fALS LINKED TO C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSIONS *A. Hübers (1), A. Volk (2), C. Kubisch (2), N. Marroquin (2), A.C. Ludolph, J.H. Weishaupt (1) ((1) Department of Neurology, (2) Department of Human Genetics, University of Ulm, Germany)*

GGGGCC hexanucleotide repeat expansions in the gene C9ORF72 have been reported to account for familial Amyotrophic lateral sclerosis (fALS) cases of European ancestry. However, scarce knowledge exists concerning the impact of an expansion on clinical characteristics in fALS patients. Moreover, information about a potential correlation of C9ORF72 hexanucleotide repeat lengths with clinical parameters is completely lacking to date. In this study, we compared detailed clinical features of patients compatible with carrying a hexanucleotide repeat expansion in C9ORF72 as determined by PCR analysis with those of patients with no known or other gene mutations in a total of 116 fALS index patients. PCR based analyses were compatible with a hexanucleotide expansion in 26 cases (22.4 %). Mean disease duration from symptom onset in these patients was substantially shorter than in patients with no known or other gene mutations. Additionally, these patients had a significantly higher proportion of bulbar onsets compared to fALS cases with no hint for a hexanucleotide expansion in C9ORF72. No significant gender differences between the two groups could be observed. First molecular results show that C9ORF72 hexanucleotide expansions may account for up to 1/5 of this large German cohort of fALS cases. Patients with molecular analyses compatible with a hexanucleotide expansion in C9ORF72 seem to show a more aggressive disease course and significantly shorter disease duration as well as a higher frequency of bulbar onset compared to patients with no known or other gene mutations. Further analyses especially Southern blot tests are needed and to determine exact repeat sizes and to confirm the diagnosis of C9ORF72 associated ALS in our cohort. Meanwhile, we will present C9ORF72 repeat lengths in our patients determined by Southern blot analyses of patient-derived lymphoblastoid cell lines.

P. 37: THE MOLECULAR BASIS OF ALS IN TURKEY *Ozoguz A1, Bilguvar K2, ParmanY3, Deymeer F3, Oflazer P3, Koc F4, Gunel M2 and Basak A N1 1Bogazici University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey 2Yale University Medical School, Department of Neurosurgery, Gunel Laboratory, New Haven, USA 3Istanbul University, Istanbul Medical School, Neurology Department, Istanbul, Turkey 4Cukurova University, Medical School, Neurology Department, Adana, Turkey*

Amyotrophic Lateral Sclerosis (ALS) is a late-onset neurodegenerative disease, characterized by death of motor neurons in the cortex, brainstem and spinal cord. Most incidences are sporadic (sALS), while 10% of cases have a family history (fALS). The genetics of ALS is complex where different modes of inheritance are observed, including autosomal dominant, autosomal dominant with reduced penetrance, autosomal recessive and X-linked inheritance. In the framework of this study, 266 Turkish ALS cases were investigated for possible mutations in several genes. Six FALS cases were shown to carry disease-causing SOD1 mutations (A4S, H71Y, N86S, D90A and L144F), while eight were carriers of the IVS-III-34 A:C polymorphism. Among these, two homozygous D90A cases, both represented as recessive, were investigated by haplotype analysis and were compared to 21 Scandinavian ALS cases in search of a common ancestry. While one patient carried the Scandinavian haplotype, the other patient displayed a different pattern. In the next step, fALS cases were analyzed for the TDP-43, FUS and ANG genes via DNA sequencing. One patient was shown to carry a 18-bp deletion at codons 143-148 of FUS, while no changes were detected in TDP-43 or ANG. The whole exome analyses of six fALS cases detected three mutations in UBQLN2 (S340I, P506S and P525S), one mutation in SPG11 (F2265L), one mutation in OPTN (ΔAA at codon 359) and one mutation in PLEKHG5 (P630H). The presence and the pattern of inheritance of these mutations were also confirmed by Sanger sequencing in patients and family members. The expected number of ALS patients in Turkey is approximately 8000. To the best of our knowledge, this is the first and the only study which compiles the results of ALS research in Turkey.

P.38: ATXN2 AND ITS NEIGHBOURING GENE SH2B3 BOTH MODULATE THE ALS RISK IN THE TURKISH POPULATION *Lahut S1, Ömür Ö1, Uyan Ö1, Ağım Z S1, Özoğuz A1, ParmanY2, Deymeer F2, Oflazer P2, Koç F3, Özçelik H4, Auburger G5 and Başak A N11Boğaziçi University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey, 2Istanbul University, Istanbul Medical School, Neurology Department, Istanbul, Turkey 3Çukurova University, Medical School, Neurology Department, Adana, Turkey 4University of Toronto, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Department of Laboratory Medicine and Pathobiology, Toronto, ON, Canada5Goethe University, Experimental Neurology, Frankfurt am Main, Germany*

Expansions of the polyglutamine (polyQ) domain (≥34) in Ataxin-2 (ATXN2) are the primary cause of spinocerebellar ataxia type 2 (SCA2). Recent studies reported that ATXN2 influences the TDP-43-dependent toxicity seen in ALS and that intermediate-length (27-33) expansions increase the risk of developing the disease in diverse populations (1-4). In this study, we aim to test the possible influence of the ATXN2 polyQ repeats on the risk of developing ALS in a Turkish cohort by genotyping 135 sporadic and 77 familial patients and

319 neurologically healthy controls. In accordance with other studies, our results confirmed that 31-32 polyQ repeats in the ATXN2 gene are associated with risk of developing ALS in 1.9% of the Turkish ALS cohort (p= 0.0261). Moreover, the ALS risk was found to be significantly associated with a 136 kb haplotype block across the ATXN2 and the neighbouring SH2B3 genes in 14.9% of our study population (p=0.0057, OR: 2.23). Our novel observations suggest that genotyping of SNPs at this locus may be useful for the study of ALS susceptibility and that ATXN2 and SH2B3 variants may interact in modulating the disease pathway.

P.39: IN-SILICO AGGREGATION PROFILE ANALYSIS OF UBQLN2 MUTATIONS IN TURKISH FALS PATIENTS Uyan *O1, Agim ZS1, Ozoguz A1, Keskin O2, Basak AN1 1Bogazici University, Molecular Biology and Genetics Department, Neurodegeneration Research Laboratory (NDAL), Istanbul, Turkey 2 Koc University, College of Engineering, Chemical and Biological Engineering, Istanbul, Turke*

To date, more than 20 different genes have been defined, which when mutated, give rise to ALS or ALS-like disease. One of the most recent and very important genes is UBQLN2, in which five different mutations have been reported in the initial study by Siddique and coworkers to result in ALS (Deng et al., 2011). Mutations in UBQLN2 are shown to cause protein aggregations in the spinal cords and hippocampi of ALS patients. Moreover, ALS cases with a C9ORF72 expansion show UBQLN2-positive cytoplasmic aggregates in the cerebellar granular layer and dystrophic neurites in the hippocampal region.In the framework of this study, three UBQLN2 mutations, identified in Turkish fALS families, S340I (novel), P506S (novel) and P525S, were subjected to in-silico aggregation profile analysis, in order to understand the effects of these mutations on ubiquilin 2 conformations. One of these mutations, S340I, is located in the central region of the protein, whereas the other two involve proline-serine changes in the PXX domain. All three mutant forms were compared to the wild-type protein and to the previously identified P497H mutant form, also in the PXX domain (Deng et al, 2011), using four different aggregation prediction tools. Ubiquilin 2, carrying the S340I and P525S mutations, was found to behave like the wild-type protein, whereas the novel P506S mutation, located in the PXX domain of ubiquilin 2, showed consistent results with the P497H form and an increased protein aggregation propensity when compared to wild-type. This study, for the first time, demonstrates that mutations in ubiquilin 2, a ubiquitin-like protein, give rise to ALS in three Turkish families. Two of these three mutations are novel, and one of them is shown in-silico to reduce the solubility of the protein when mutated. A validation of this finding by autopsy specimen or cell culture modeling would enhance the significance of this result.

P.40: EFFECT OF FUMARIC ACID ESTERS ON THE HIF-1alpha MEDIATED RESPONSE

Diana Wiesner, Judith Eschbach, A.C. Ludolph, Anke Witting, Luc Dupuis (University of Ulm, Germany)

Fumaric acid esters (FAE) are oral analogs of fumarate and have been used in the treatment of psoriasis in Europe for more than 50 years (Arbiser, 2011). But the mechanism is still unknown. Indeed, intermediates of the Citric Acid Cycle, including fumarate, are known inhibitors of Proline Hydroxylases (PHD) (Serra-Pérez et al., 2010) leading to stabilization of HIF1 under normoxic conditions (Koivunen et al., 2006). This so called pseudo hypoxic response leads to an upregulation of VEGF with proven neuroprotective potential in

neurodegenerative diseases. In a search for underlying mechanisms, we hypothesize that an elicited pseudo hypoxic response in Amyotrophic lateral sclerosis, caused by Fumaric acid esters, might be protective through neuroprotective and metabolic effects. To investigate this we focused on astrocytes, largely involved in Amyotrophic lateral sclerosis pathogenesis and - in vivo - a major source of vascular endothelial growth factor (VEGF) (Choi et al., 2010; Huang et al., 2010). We treated astrocytes isolated from transgenic high copy B6.Cg-Tg(SOD-G93A) mice with dimethylfumaric (DMF) and diethylfumaric (DEF); astrocytes from wildtype littermates served as controles. We found an increase of HIF-1alpha expression and also an increased VEGF release in cell culture media. These studies demonstrate an beneficial effect raised by Fumaric acid esters inducing pseudo hypoxic response, which might be especially relevant for Amyotrophic lateral sclerosis.

P.41: UPREGULATION OF PDI IN ALS MICROGLIA AND ER STRESS DEPENDENT ACTIVATION OF NADPH OXIDASE. *Ms. Merja Jaronen (Dept. of Neurobiol., A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland)*

Protein-disulphide isomerase (PDI) is a chaperone in the endoplasmic reticulum assisting oxidative protein folding in all types of cells, including neurons and glia. In neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), up-regulation of PDI is thought to be an important part of unfolded protein response (UPR) that is considered as an adaption reaction to protect the neurons. In G93A-SOD1 ALS mice our results showed that even though PDI expression was high in motoneurons, the PDI positive area was not changed at early symptomatic age and we detected only slightly increased motoneuronal PDI expression when compared to wild type (wt) mice. In contrast, we found a striking up-regulation of PDI in the spinal cord microglia of G93A-SOD1 mice, indicating that UPR may take place not only in neurons but also in glial cells in ALS. Because mutant SOD1 has been reported to cause sustained activation of NADPH oxidase (NOX) in microglia, we investigated the role of PDI and UPR in NOX activation. In BV-2 microglia UPR resulted in NOX activation coupled with increased superoxide production and TNF-α release. The phenomenon was recapitulated in primary rat microglia, murine macrophages and human monocytes. Importantly, pharmacological inhibition of PDI or its down-regulation by siRNAs prevented NOX activation in microglia and subsequent production of superoxide. Thus, increased PDI activity in microglia may exacerbate oxidative stress through NOX activation and contribute to neurotoxicity, including the motor neuron degeneration in early stages of ALS.

P.42: EFFECT OF PPARs AND THEIR CO-ACTIVATORS ON THE ALTERNATIVE ACTIVATION OF MICROGLIA *Hanna Bayer, Anne Buttgereit, Diana Wiesner, Patrick Weydt und Anke Witting (Department of Neurology, Ulm University, Germany)*

Amyotrophic lateral sclerosis is associated with mitochondrial damage and activated microglia. We have shown previously that dysfunctional mitochondria inhibit the beneficial alternative (M2) activation of microglia. Our preliminary data suggests that this alternative activation is also inhibited in microglia isolated from the SOD-1 mouse model of ALS. It has been shown that the alternative activation is associated with the activation of PPARs and especially with the activation of the co-activator PGC-1beta. PPAR gamma and PGC-1 alpha have been shown to modify ALS. Our aim was therefore to identify the role of the different PPAR receptors (alpha, delta and gamma) and their co-activators on the alternative activation. We stimulated primary mouse microglia with specific PPAR agonists and

antagonists and investigated their effect on the anti-inflammatory response associated with the Interleukin-4 (IL-4) induced alternative activation. Furthermore we investigated the effect of PPAR and/or IL-4 on the expression and activation of PGC-1alpha and beta. We found that especially PPAR alpha and PPAR delta stimulated the alternative activation, whereas PPAR gamma especially stimulated the alternative activation in PGC-1alpha knock out microglia. Furthermore we found that IL-4 induced the expression of PGC-1beta. PGC-1alpha was only barely detectable in microglia. Very preliminary results suggest that PPAR gamma induces the expression of PGC-1alpha, whereas it inhibits the expression of PGC-1beta. Our results suggest that PGC-1beta is involved in mediating the beneficial effects of the alternative activation, whereas PGC-1alpha might interfere with this beneficial effect. To reconstitute the alternative activation of microglia in ALS it might be therefore better to use PPAR alpha of delta agonist instead of PPAR gamma agonists. This work is supported by a grant from the Latran Foundation to P.W.

P.43: KINESINS EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF HUMANS AND TRANSGENIC MICE WITH hSOD1G93A *Magdalena Kuzma-Kozakiewicz, Agnieszka Chudy, Ewa Usarek, Beata Gajewska, Anna Baranczyk-Kuzma (Department of Neurology, Department of Biochemistry, Medical University of Warsaw, Poland)*

Background: Dysfunction of fast axonal transport may lead to motor neurons degeneration. The aim of the study was to investigate the expression of kinesins (KIFs) involved in the anterograde (KIF5A, 5C) and the retrograde (KIFC3/C2) axonal transport in the CNS from humans and transgenic mice.

Methods: The KIFs expression was studied real-time qPCR and RT-PCR in patients who died of sporadic amyotrophic lateral sclerosis (ALS) and from transgenic mice with human SOD1G93A-associated ALS, at presymptomatic and symptomatic stages.

Results: KIFs expression in human motor cortex of individual ALS subjects was higher than in adjacent sensory cortex, in contrary to the expression in control brains. It was also significantly higher in frontal cortex of symptomatic, but not presymptomatic mice compared to wild-type controls. However, the mean KIFs expression in human ALS motor and sensory brain cortex was lower than in control cortexes. To a lower extend the decrease of KIFs mean expression also occurred in human but not in mice ALS spinal cords and in both human and mice cerebellum.

Conclusion: Disturbances in KIFs expression in the CNS of sporadic and SOD1G93A-mediated ALS may affect both anterograde and retrograde transport, especially in the brain cortex.

P.44: HDAC6 INHIBITION RESTORES THE PHENOTYPE OF NEW MOUSE MODLES OF CHARCOT-MARIE-TOOTH DISEASE *Constantin D'Ydewalle (Vesalius Research Center, Laboratory of Neurobiology, VIB, KU Leuven, Leuven, Belgium*

Charcot-marie-tooth disease (CMT) is the most common inherited disorder of the peripheral nervous system affecting 1 in 2,500 individuals. CMT patients show progressive and length-dependent muscle weakness and atrophy, foot and hand deformities, and steppage gait. Depending on the severity of the disease, patients become wheelchair-bound. Over 50 different genes have been associated with CMT. Several disease-linked missense mutations have been identified in the gene encoding the small 27-kDa heat-shock protein B1 (HSPB1). To investigate the mechanism underlying mutant HSPB1-induced CMT, we developed and characterized transgenic mice that express wild type (WT) or mutant (p.S135F or p.P182L) HSPB1 in neurons. Both mutant HSPB1 mice displayed progressive motor defects due to

muscle atrophy caused by axonal loss. Furthermore, mice expressing mutant HSPB1 developed hind paw deformities and an altered gait. While p.S135F-HSPB1 mice showed sensory defects, p.P182L-HSPB1 mice did not. We found a dramatic decrease in mitochondrial motility in neurons isolated from adult mutant HSPB1 mice. This was caused by a reduction of acetylated tubulin abundance in peripheral nerves isolated from symptomatic mutant HSPB1 mice. Finally, we treated symptomatic mutant HSPB1 mice with inhibitors that target the tubulin deacetylating enzyme called histone deacetylase 6 (HDAC6). After 3 weeks of treatment, several key features of the phenotype of mutant HSPB1 mice were restored due to increased tubulin acetylation. Finally, HDAC6 inhibition also reversed the axonal transport defects after the treatment period. In conclusion, we developed transgenic mice that selectively express mutant HSPB1 in neurons. Both mutant HSPB1 mice phenocopy human conditions dependent on the mutation expressed. HDAC6 inhibition restored the phenotype at the behavioral and functional level. Targeting HDAC6 offers perspectives as a potential therapy for inherited peripheral neuropathies as well as for neurodegenerative disorders characterized by axonal transport defects.

P.45: GENDER-SPECIFIC MECHANISM OF SYNAPTIC IMPAIRMENT AND ITS PREVENTION BY G-CSF IN A MOUSE MODEL OF ALS *Eveliina Pollari (Dept. of Neurobiol., A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland)*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of motoneurons which progresses differentially in males and females for unknown reason. In this study we measured electrophysiologically pre- and post-synaptic properties of neuromuscular junction in the diaphragm of G93A-SOD1 mouse model of ALS at early symptomatic stage. Furthermore, we wanted to determine whether sex or anti-inflammatory treatment with granulocyte colony stimulating factor (G-CSF) alters the properties of synaptic transmission or level of reactive oxygen species (ROS) in the spinal cord. Miniature and evoked endplate potentials (MEPPs and EPPs) were recorded in the diaphragm muscle using intracellular microelectrode technique. Electrophysiological testing revealed that in G93A-SOD1 mice the postsynaptic function was mainly preserved since the amplitude of MEPPs was not altered. Conversely, the presynaptic properties were greatly affected by the disease as observed by dramatically reduced probability of spontaneous acetylcholine release. In males, but not females, this was accompanied by reduced readily releasable transmitter pool. Transmitter release in both sexes retained sensitivity to the inhibitory action of ROS, whereas the production of ROS was increased only in the spinal cords of male G93A-SOD1 mice. Previously we have shown that treatment with G-CSF prolongs survival of G93A-SOD1 males and here we show it also attenuates the ROS production indicating involvement of the antioxidant mechanisms. In male G93A-SOD1 mice G-CSF treatment improved the presynaptic properties. Consistent with our findings at the synaptic level, G-CSF did not change the survival of female ALS mice. This is the first detailed electrophysiological analysis of impaired synaptic function in a mouse model of ALS providing a sensitive surrogate marker for ALS research. Our results support the previous findings of sexual dimorphism in ALS progression and drug response.

P.46: TDP-43 DYSFUNCTION CAUSES SYNAPTIC AND SUBSEQUENT AGE-RELATED NEURODEGENERATION IN DROSOPHILA Danielle C. Diaper1,7, Yoshitsugu Adachi1,7, Ben Sutcliffe2, Dickon M. Humphrey1, Chris Elliott3, Triona Fielding1, Mubarik Burki1, Zoe N. Ludlow1, Lies Vanden Broeck4, Bart Dermaut4,5, Patrick Callaerts4, Ammar Al-Chalabi6, Christopher E. Shaw6, Iain Robinson2 & Frank Hirth11Department of Neuroscience, Institute of Psychiatry, King's College London, London/United Kingdom; 2Institute of Biomedical & Clinical Science, University of Plymouth, Plymouth/United Kingdom;3Department of Biology, University of York, York/United Kingdom;4Laboratory of Behavioural & Developmental Genetics, Centre for Human Genetics, University of Leuven, Leuven/Belgium & VIB Centre for the Biology of Disease, Leuven/Belgium;5INSERM U744, Pasteur Institute of Lille, University of Lille North of France, Lille/France.6Department of Clinical Neuroscience, Institute of Psychiatry, King's College London, London/United Kingdom;

Cytoplasmic accumulation and mutation of TAR DNA binding protein 43 (TDP-43) characterise familial and sporadic forms of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), suggesting that TDP-43 dysfunction is causally related to disease formation. However, the underlying mechanisms are unknown. Here we show that dysfunctional Drosophila TDP-43, TAR DNA Binding Protein Homolog (TBPH), causes age-related neurodegeneration. Both loss and gain of TBPH severely affect development and result in premature lethality. Affected flies are characterized by synaptic deficits rather than structural alterations of the neuromuscular junction, and exhibit severely impaired motor behavior. Prolonged dysfunction of TBPH is followed by trans-synaptic defects and in turn the age-related and progressive degeneration of neurons involved in the control of motor behavior. Moreover, we found that necrotic cell death rather than TBPH-mediated activation of programmed cell death underlies age-related neurodegeneration. These findings demonstrate that both loss and gain of function mechanisms are causally related to, and equally contribute to impaired neuronal viability and identify a TDP43-mediated pathogenic pathway whereby synaptic deficits cause defective motor behavior, and in turn progressive deconstruction of neuronal connections, ultimately leading to age-related neurodegeneration.

P.47: POTENTIAL EFFECTS OF LOW-FREQUENCY MAGENTIC FIELDS (LF-MFs) ON AMYOTROPHIC LATERAL SCLEROSIS *Martina Liebl (1), Andreas Horn (1), Blanka Pophof (2), Christian Behl (1), Albrecht M. Clement (1) (Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg University Mainz)1. Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg University Mainz, Germany 2. Federal Office for Radiation Protection, Salzgitter,Germany*

ALS is a devastating neurological disorder that leads to paralysis of the patient due to selective death of motoneurons in the spinal cord and the motor cortex. Although some ALS cases are familial, the vast majority of cases is sporadic. Therefore environmental factors are discussed as potential risk factors. Several epidemiological studies investigated whether the exposure to low-frequency magnetic fields (LF-MFs) increases the risk to develop ALS. Some of these studies found a positive correlation between LF-MF exposure and disease. LF-MFs are produced by electrical current flow and can penetrate biological tissue. Some people, due to their habitation in close proximity to overhead transmission lines, or due to their profession as train drivers, workers in electrical properties etc. are exposed to increased amounts of LF-MFs, often over a long time period. To investigate if LF-MFs interfere with molecular pathways of mutant SOD1-toxicity, we compare the disease course of large

cohorts of SOD G85R and SOD G93A mice exposed to LF-MF (50Hz, 1mT) to sham exposed animals. We examine potential effects of LF-MFs on the life span and the disease duration. Furthermore we compare protein levels, RNA-levels, markers for oxidative damage, glial activation and enzymatic activities between exposed and sham exposed mice to detect a potential influence of LF-MFs on ALS. This project is funded by the Federal Office for Radiation Protection

P.48: COMMON EARLY ALTERATIONS IN LUMBAR MOTONEURONS OF SOD1G93A AND SOD1G85R JUVENILE MICE *Jacques Durand, Anton Filipchuk, Arnaud Pambo Pambo, Sylvie Liabeuf, Cecile Brocard, J.P. Gueritaud (CNRS, UMR 7289, Aix Marseille Université, Institut de Neuroscience de la Timone, Marseille, France)*

In superoxide dismutase 1 (SOD1) mutant mice, the standard animal model of familial Amyotrophic Lateral Sclerosis (ALS), it has been shown that spinal motoneurons have altered electrical and morphological properties at an early postnatal age. In the present study we examined the morphology and the electrical properties of lumbar motoneurons in two strains of mutant mice (SOD1G93A and SOD1G85R) to determine common postnatal alterations. Why neonate motoneurons grow abnormally and which is the link between these early abnormalities and the late motoneuronal degeneration are still open questions. In the whole isolated brainstem/spinal cord preparations, we recorded lumbar motoneurons in wild type (WT) and in SOD1 juvenile mice using intracellular recording and staining. Following histological procedures, the labeled motoneurons were reconstructed in 3D using NeurolucidaTM system for morphometric and topologic parameters analyzis. We used computer models of 3D reconstructed WT and SOD1 motoneurons to assess the functional consequences of the morphological changes. In another set of experiments, we also used specific antibodies directed against K+/Cl- co-transporter KCC2, and NR2A and GLUR1 subunits of NMDA and AMPA receptors, respectively. Immunoblotting was performed to determine the protein expression in the whole lumbar cords. These experiments revealed that both SOD1G93A and SOD1G85R motoneurons exhibit a higher number of dendritic branches at postnatal days P8-P9 as compared to non transgenic WT motoneurons and develop a precocious hypoexcitability with a lower gain of the discharge frequency-intensity curves probably due to variation of synaptic input. Furthermore western blot analysis of NR2A subunit and KCC2 expressions in lumbar segments revealed differences between WT and SOD1 mice at post natal days corresponding to the morphological dendritic changes. These results demonstrate several common abnormalities in the two SOD1 mutant strains and suggest very early imbalance of afferent inputs.

P.49: NEGATIVE RESULTS: CITICOLINE IS NOT PROTECTIVE IN THE SOD1 (G93A) MOUSE MODEL OF ALS *Dr. Sarah Knippenberg (Hannover Medical School - Neurology)*

Introduction: Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease with glutamate excitotoxicity as one of several factors contributing to the death of motor neurons. The only FDA approved drug for ALS (riluzole) with at least marginal therapeutic efficiency modulates glutamatergic neurotransmission (Bensimon et al., 1994). Efficient reduction of glutamate-induced excitotoxicity therefore still is among the most promising strategies to counteract death of motor neurons in ALS. Cytidine 5-diphosphocholine (citicoline) has been shown to have beneficial effects in a variety of neurological disorders, mainly based on its anti-glutamatergic activity.

Objectives: We therefore assessed the neuroprotective potential of citicoline in the SOD1 (G93A) transgenic mouse model for Amyotrophic Lateral Sclerosis (ALS).
Methods: We administered citicoline to transgenic SOD1 (G93A) mice via intraperitoneal (i.p.) injection starting before disease onset (day 30). By monitoring of survival, motor function, weight and general condition we examined possible therapeutic effects. Additional animals were used for histological studies to analyse the effect of citicoline on motoneuron survival, astrocytosis and myelination.
Results: Daily injections of citicoline neither improved motor function nor prolonged survival of the animals. Histological assessment showed no effect of citicoline treatment on the number of surviving motor neurons or the extent of astrocytosis and myelination in the spinal cord. Thus, citicoline seems to be not protective in the SOD1 (G93A) mouse model for ALS. Keywords: SOD1 (G93A) mouse model, citicoline, behavioural assessment, histological analyses.

P.50: USE OF MAGNETIC RESONANCE IMAGING TO MONITOR DISEASE PROGRESSION IN ANIMAL MODELS OF FAMILIAL ALS *Ilaria Caron (Mario Negri Institute for Pharmacological Research), Micotti E., Plebani, L., Merlino G. and Bendotti C*

Introduction: ALS is a progressive neurodegenerative disease caused by degeneration of upper and lower motoneurons, leading to rapid progressive muscles wasting and respiratory failure within few years from symptoms onset. The disease phenotype is quite heterogeneous, making difficult a defined prognosis. MRI has been a useful tool to recognize ALS by excluding other ALS mimic syndromes and is now considered a promising tool for monitoring the disease progression and assess the therapeutic effect of neuroprotective agents.
Methods: We used T2 maps to evaluate longitudinal degeneration of cranial motor nuclei of mutant SOD1 mice, a reliable model of fALS, and Diffusion Tensor Imaging (DTI) to study the mice lumbar spinal cord ex vivo. We then compared MRI results with the histopathological analysis of cranial motoneurons and lumbar spinal cord axons at different stages of disease.
Results: We observed an hyperintensity of T2-weighted images in SOD1G93A mice, corresponding to Trigeminal and Facial nuclei, already at the pre-symptomatic stage. At this phase of the disease histopathological analysis revealed vacuoles formation in these nuclei but not cell loss. These data confirm the powerful of MRI in detecting nuclei degeneration before the loss of motoneurons. In addition, using DTI, we observed first signs of axonal impairment starting at the symptomatic stage of the disease in the ex-vivo spinal cord of SOD1 mutant in respect to wild type mice, reproducing results reported in ALS patients. The axonal loss has been confirmed by histological analysis using SMI-31 marker.
Conclusion: These data indicate that MRI analysis is predictive of the histopathological changes of lumbar spinal cord white matter and of cranial motor nuclei during disease progression in mouse model of fALS.

P.51: PRE-SYMPTOMATIC NEUROINFLAMMATORY INSIGHTS FROM MULTIMODAL MRI IN THE SOD1 MURINE MODEL OF ALS Evans MC, Stolp HB, Anthony D, Talbot K, Sibson N, Turner MR (University of Oxford)

Background: Studying the earliest, pre-symptomatic pathological changes in human ALS is challenging. T2-weighted MRI changes in brainstem motor nuclei of the transgenic SOD1 murine model of ALS support neuroinflammatory mechanisms in pathogenesis. However, the time-course and biological underpinnings of such changes remain poorly understood.

Methods: MRI was performed at 7T in 4 mutant and 4 wild-type SOD1 mice at 40, 60, 80, 100 or 120 days. In addition to T2-weighted imaging, Pre- and post-Gd-DTPA T1-weighted images were acquired to test blood-brain barrier (BBB) permeability. 'Molecular MRI', using a T2*-weighted sequence sensitive to intravenously injected VCAM-1-targeted microparticles of iron oxide (MPIO), was used to investigate VCAM-1 up-regulation. In an additional cohort of mice at 120 days, magnetization transfer ratio (MTR) maps and diffusion-weighted imaging (DWI) were used to identify further MRI-detectable changes and assess their correlation with T2 signal changes. Cross-sectional immunohistochemistry and qRT-PCR for cellular adhesion molecules were performed.
Results: Age-dependent T2 hyperintensities were observed in brainstem nuclei V, VII and XII. These increases in T2 were visible pre-symptomatically, from 60 days, which is considerably earlier than previously reported. No evidence of BBB breakdown was found at any time-point, nor was VCAM-1 upregulation observed using either VCAM-1-targeted MRI or immunohistochemistry. Instead, upregulation of ICAM-1 was evident from 100 days both immunohistochemically and by RT-PCR. DWI and MTR maps both revealed effects of pathology at 120 days, but to a lesser extent than T2 relaxation.
Conclusions: CNS lymphocyte infiltration in ALS reported in other studies does not seem to be VCAM-1 dependent. Given their differential sensitivities, T2-weighted, MTR and DWI changes may be sensitive to distinct aspects of neuroinflammatory pathology. Multimodal MRI is able to probe rodent models of ALS and reveal pre-symptomatic changes that have not previously been reported. Ultimately, these methods may identify novel targets for therapy.

P.52: MOBILIZATION OF HEMATOPOIETIC BONE MARROW STEM CELLS BY G-CSF IS NOT PROTECTIVE WHEN INDUCED IN SYMPTOMATIC SOD1G93A MOUSE MODELS

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Introduction: We explored the possibility that activation of bone marrow(BM) stem cells by Granulocyte colony-stimulating factor(G-CSF) could be applied as therapy in mouse models of familial ALS.
Methods: We used two strains of transgenic SOD1G93A mice: 129SvG93A mice showing an early onset and faster disease progression compared to C57BL/6G93A mice. We examined the effect of 10 days treatment with G-CSF 300 µg/Kg/day/subcutaneously, starting at the onset of motor symptoms in both mouse strains. All mice received also Edu 50mg/kg/day/intraperitoneally to identify the proliferating cells into the CNS. One group of mice were used to evaluate the effect of treatment on disease progression and survival; another was examined for histopathological analysis and proliferating cells counting and characterization 2 days after treatment.
Results: GCSF induced an increase of total blood nucleated cells in both SOD1G93A mouse strains. However, while no effect was found in the disease progression of C57BL/6G93A mice, the motor impairment and survival of 129SvG93A mice was worsened. Edu+ cells were remarkably increased in the lumbar spinal cord of both mouse strains in respect to respective non transgenic littermates, with a larger effect seen in 129SvG93A mice. EdU co-localize in both strain with markers of oligodendrocyte precursors and mature cells, macrophage-microglia and astrocytes although the proportions of these cellular phenotype remarkably differed between the two strains. However, the GCSF induced a significant

increase of Edu+ cells only in the lumbar spinal cord white matter of 129SvG93A mice and most of them exhibited a macrophage-microglia phenotype.
Conclusion: These data indicate that activation of BM-derived myeloid progenitors by G-CSF in SOD1G93A mice at the onset of symptoms has no protective effect. On the contrary, in a mouse model with a more rapid disease course the cytokine displayed a detrimental effect which may be due to increased recruitment of macrophage-microglia with toxic properties.

P.53: IS DEFECTIVE MITOCHONDRIAL Ca2+ STORAGE IS A PRIMARY CONTRIBUTOR TONEURONAL DEGENERATION? *P. Parone Department of Cell Biology, University of Geneva, Geneva, Switzerland.*

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal adult-onset neurodegenerative disorder, characterized by selective loss of motor neurons and skeletal muscle degeneration. An early event is thought to be denervation-induced muscle atrophy accompanied by alterations in mitochondrial activity and morphology within muscle. The transcriptional co-activator PGC-1a induces multiple effects on muscle, including increased mitochondrial mass and activity. We elevated PGC-1a levels using a transgenic approach in muscles of mice that develop fatal paralysis from an ALS-causing SOD1. This promoted an increase in mitochondrial mass and activity throughout disease course, together with the activation of other PGC1a-dependent pathways, and was accompanied by retention of muscle function, delayed muscle atrophy and significantly improved muscle endurance even at late disease stages. However, muscle denervation and motor neuron degeneration were unaffected and survival was not extended. This evidence demonstrates that mitochondrial dysfunction within muscle does not contribute to disease progression and that muscle is not a primary target of mutant SOD1 mediated toxicity, but that drugs increasing PGC-1a activity and mitochondrial activity in muscle represent an attractive therapy for maintaining muscle function in patients during progression of ALS.

P.54: SOD1 mRNA ARE SEQUENCES AS NOVEL TARGETS FOR ELAV-MEDIATED POST-TRANSCRIPTIONAL MODULATION: A NEW CLUE FOR ALS PATHOGENESIS?
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Studies to date on SOD1 gene are mostly focused on alterations in the coding region and their associations with ALS.
However, the importance to study SOD1 expression regulation at multiple levels is becoming clear, since altered SOD1 induction may be involved in ALS, as demonstrated by recent data showing increased SOD1 transcript levels in pathological tissues of sporadic ALS patients compared to control subjects (Gagliardi et al., 2010). Even if SOD1 has been often considered a "housekeeping gene" due to its high and ubiquitous expression, it is now clear that its induction is fine-tuned modulated by complex intracellular mechanisms which probably involve both transcriptional and post-transcriptional events. With regard to the post-transcriptional regulation of SOD1 expression, in 1995 Kilk et al. identified some adenine/uridine-rich stretches in the 3'UTR of SOD1 mRNA, and hypothesized that these sequences may affect the expression of the correspondent protein. In general, the Adenine/uracil-Rich Elements (ARE) represent indeed the docking sites for many RNA-

binding proteins (RBPs), which can influence one or more steps of the metabolism of the target transcripts (Chen, 1995). Among the ARE-binding RBPs, a relevant place is taken by ELAV-like proteins (Antic, 1997). ELAV-like proteins act mainly as positive regulators of gene expression, since they can increase the stability and/or translation of target mRNAs whose corresponding proteins are fundamental for key cellular functions.
Within this context, in a cellular model, the human SH-SY5Y cells, we first investigated whether SOD1 mRNA represents a target of ELAV-like proteins, and whether the binding between ELAV-like proteins and SOD1 mRNA, and consequently SOD1 protein expression, are favoured by oxidative stress, a condition observed in ALS.
By performing RNA Electrophoretic Mobility Shift Assay (REMSA) and RNA-immunoprecipitation (RNA-IP) assay, we proved the molecular interaction between ELAV proteins and SOD1 mRNA and we also observed that the cell treatment with 1mM H_2O_2 for 30 min induced a significant increase (about 5-fold versus control) of the binding between ELAV proteins and SOD1 mRNA. Given that ELAV proteins can form ribonucleoproteic (mRNP) complexes that associate to the translational machinery, and have been demonstrated to enhance translation of some target mRNAs (Amadio M. Et al., 2008), we tested whether the treatment with H_2O_2 also influenced SOD1 protein expression. Western blotting experiments showed an increased SOD1 protein level in mRNP complexes after the exposure. We demonstrated in our cellular model that SOD1 mRNA is a target of ELAV proteins, we decided to investigate whether also ELAV proteins expression and/or activation are affected by SALS disease. Specifically, considering that ELAV proteins can be activated by phosphorylation (Amadio et al., 2008), we isolated ELAV/HuR protein by performing an immunoprecipitation on the cytoplasmic fraction of PBMCs from control and SALS subjects, and analyzed its possible phosphorylation. Notably, we found an increase in both serine and threonine phosphorylation of ELAV/HuR protein in SALS samples with respect to controls, suggesting that this protein is more activated in PBMCs from SALS patients. Furthermore, we wanted to investigate possible changes of ELAV proteins expression in the cerebral area specifically involved by SALS: the motor cortex. We thus evaluated ELAV proteins expression and intracellular localization by performing immunohistochemistry (IHC) analysis in the motor cortical tissues from SALS patients and healthy controls. The ELAV-positive signal was much more intense in the cerebral sections from SALS patients than in the ones from control subjects. Moreover, in the control tissues ELAV proteins appeared mainly confined to the nucleus, while in the sections from SALS subjects the same proteins were strongly localized also in the perinuclear region and in the cytoplasm.
These data suggest that the up-regulated RNA-binding proteins may be more available and massively enrolled in the positive regulation of target mRNAs, such as SOD1. In support of this hypothesis, it is worth of note that SOD1 mRNA levels are significantly increased in the motor cortex from SALS patients in comparison to controls, as resulted by quantitative real time RT-PCR analysis.
These studies shed new light on SOD1 gene expression regulation, suggesting the existence of novel molecular cascades in cellular response to oxidative stress and they will try to disclose new mechanisms underlying ALS aetiology, thus identifying potential innovative therapeutic strategies for this disease.

P.55: SENSING ION CHANNELS (ASICS) CONTRIBUTE TO MOTONEURON DEGENERATION IN AN ANIMAL MODEL OF ALS *Aine T Behan#, Bridget Breen#, Ina Woods, Karen Coughlan, Mollie Mitchem, Jochen H.M.Prehn (Department of Physiology and Medical Physics, Centre for the Study of Neurological Dis orders, RCSI) Dublin Ireland*

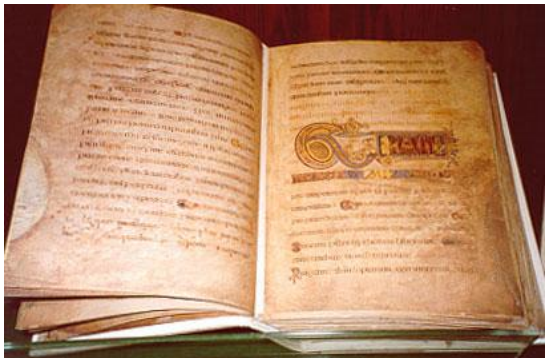
Amyotrophic lateral sclerosis (ALS) is a fatal neurological condition with no cure. Mitochondrial dysfunction, Ca2+ overloading, and a local hypoxic/ischemic environment have been implicated in the pathophysiology of ALS and are conditions that may initiate metabolic acidosis in the affected tissue. We tested the hypothesis that acidosis, and, in particular acid-sensing ion channels (ASICs), are involved in the pathophysiology of ALS. By Neutral Red assessment, we found that acidosis increased across disease progression in the spinal cord of SOD1 ALS mice. Moreover, motoneurons were selectively vulnerable to acidotoxicity in vitro. Acidotoxicity was partially reduced in asic1a-deficient motoneuron cultures, and crossbreeding of SOD1 with asic1a-deficient mice delayed the onset and progression of motor dysfunction in SOD1 mice. Interestingly, we also noted a strong increase in ASIC2 expression in motoneurons of SOD1 mice and sporadic ALS patients during disease progression. Pharmacological pan-inhibition of ASIC channels with lipophilic amiloride derivative, 5-(N,N-Dimethyl) amiloride hydrochloride, potently protected cultured motoneurons against acidotoxicity, and, given post-symptom onset, significantly improved lifespan, motor performance and motoneuron survival in SOD1 mice. Together, our data provide strong evidence for the involvement of ASIC channels in motoneuron degeneration, and highlight the potential of ASIC inhibitors as a new treatment approach for ALS. This research was supported by grants from Science Foundation Ireland (08/IN1/1949) and by a grant from the Health Research Board (HRA_POR/2011/108).

P.56: ANDROGENIC/ ANABOLIC STEROID DYSREGULATION AS RISK FACTOR FOR AMYOTROPHIC LATERAL SCLEROSIS *T. Aggarwal (1), M. Galbiati (2), A. Poletti (3), M. Pennuto (1) ((1) Department of Neuroscience, Istituto Italiano di Tecnologia, Genoa, Italy) (1) Department of Neuroscience, Istituto Italiano di Tecnologia, Genoa, Italy (2) Dipartimento di Endocrinologia, Fisiopatologia e Biologia Applicata, and Centre of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, Milan, Italy (3) DEFIB/CEND-Università degli Studi di Milano, Italy*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by the selective loss of upper and lower motor neurons and skeletal muscle atrophy. ALS is characterised by wasting and paralysis of skeletal muscle with death of patients occurring in about three to five years from diagnosis. It has previously been shown that muscle plays an important role in the pathogenesis of ALS and other neuromuscular diseases, such as spinal and bulbar muscular atrophy (SBMA). Interestingly, SBMA is caused by a mutation in the androgen receptor, suggesting that proper androgen signalling and androgen receptor function are critical to motor neuron survival and muscle homeostasis. The incidence of sporadic ALS (sALS) is higher in males than in females, and it declines with age, when androgen levels in the serum decrease. Moreover, varsity athletes, army members, and Italian football players are at high risk to develop ALS. Male steroids, such as testosterone and the synthetic anabolic steroid nandrolone, are used as performance enhancing agents, suggesting a

possible link between sALS and androgen signalling. In order to assess the role of androgens on ALS pathogenesis, we treated a mouse model of ALS expressing mutant SOD1 with nandrolone. Preliminary evidence indicates that treatment of these mice with nandrolone affects disease pathogenesis (body weight loss, motor dysfunction and survival). This research will shed light onto the role of androgens in sALS with identification of androgens and androgen receptor as novel risk factors for sALS. This can lead to opening new therapeutic avenues for prevention and treatment of sALS.

SOCIAL EVENTS

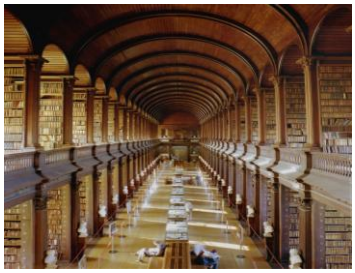


Visit to the Old Library and Historic Book of Kells

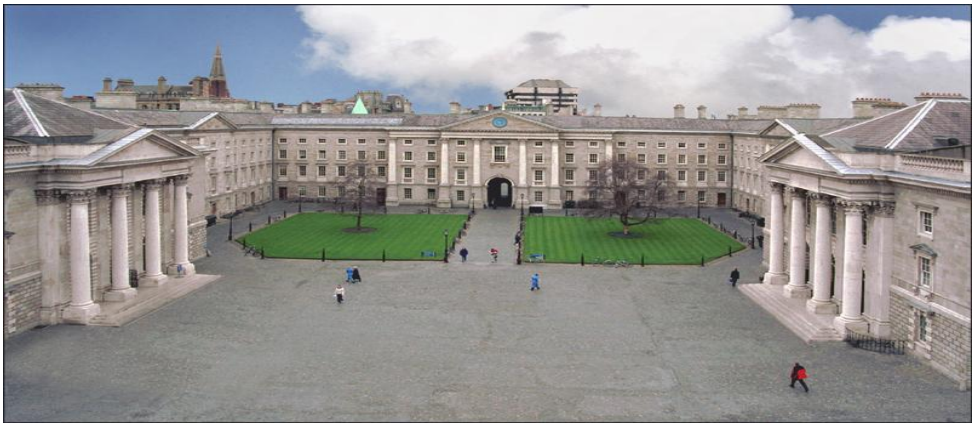
(Tickets included in delegate pack)

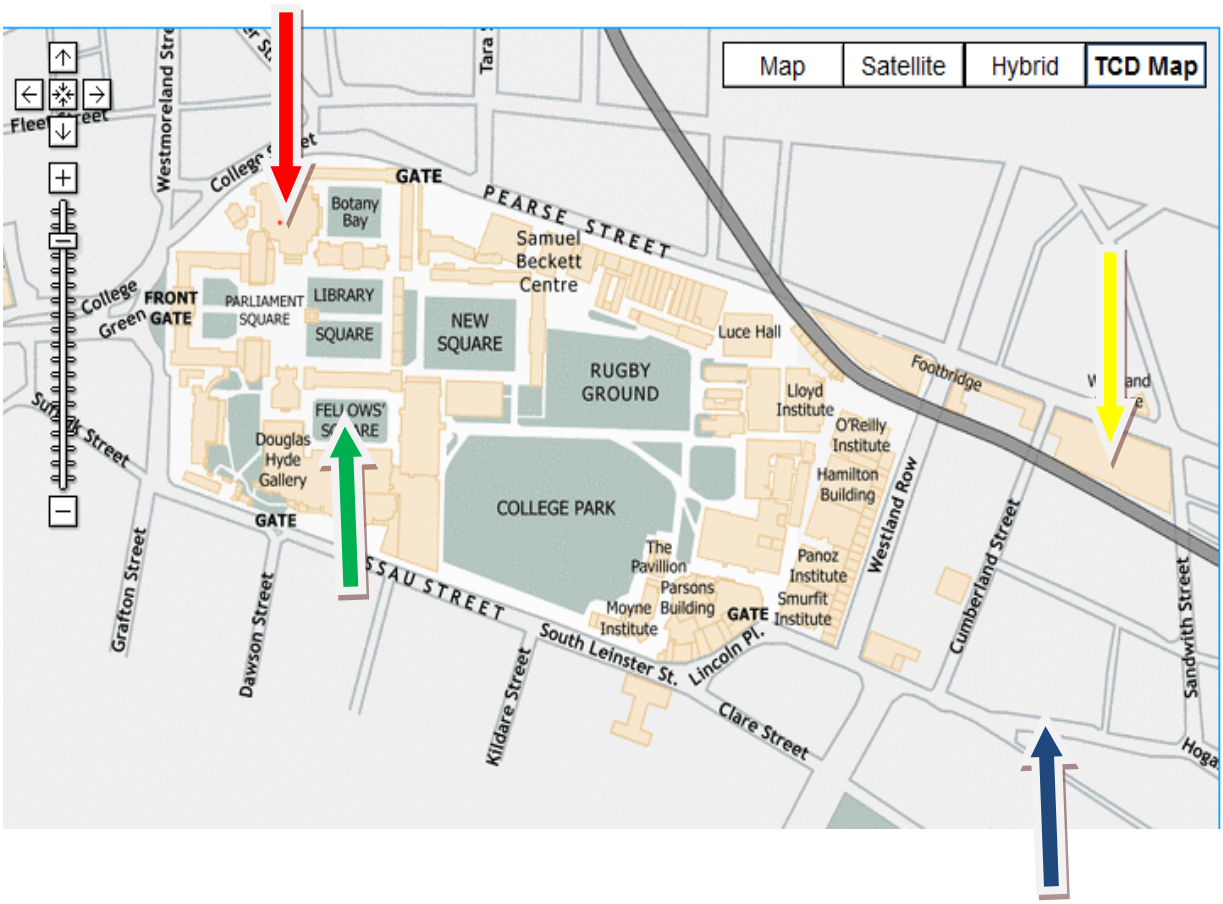
The Book of Kells was written by Irish monks in 800AD on the Scottish island of Iona, and transported to Kells in Ireland for safe keeping during the Viking raids. It has been on display in the Old Library at Trinity College Dublin from the mid 19th century.

Opening Hours: Monday-Saturday 9.30-17.00
Sunday 09.30-16.30
See Map page 68



Saturday, 26th May 2012
20.00
GALA Dinner in the Dining Hall
Front Square Trinity College





MAP OF CAMPUS

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