ALS and other motor neuron diseases (except SMA) #2299

**P02-18** Genetics of Amyotrophic Lateral Sclerosis (ALS) disease in Iran

Afagh Alavi (1), Elahe Elahi (1), Shahnour Nafissi (2), Mohammad Rohani (3), Hosein Shamshiri (2)
1. No, School of Biology, College of Science, University of Tehran, Tehran, Iran, Tehran, Iran
2. No, Department of Neurology, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran
3. No, Department of Neurology, Iran University of Medical Sciences, Hazrat Rasool Hospital, Tehran, Iran, Tehran, Iran

Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease. It is generally adult onset and fatal. ALS usually appears sporadic (SALS), but 1%-13% of cases are familial (FALS). Today, more than 20 causative genes are known, and mutations in most are very rare. Based on functions of the genes, oxidative stress, axonal transport, vesicular transport, protein aggregation, and RNA metabolism are relevant to ALS pathology. Importantly, mutations in various ALS genes have also been observed in other neurodegenerative diseases and it is thought that understanding the pathology of ALS will contribute to understandings of other disorders. We for the first time studied the genetic basis of ALS in 107 Iranian patients. Average age at onset in our cohort was relatively young and survival was long. Linkage analysis in four families using high density SNP microarrays led to identification of a locus that included the well-known SOD1 gene in three of the families. Mutation screening identified the common p.Asp90Ala mutation in SOD1 in the three families. Subsequent screening of SOD1 responsible for slowly progressive autosomal recessive ALS with juvenile-onset.

**P02-19** Demographic and clinical features of ALS in northeastern Iran from March 2007 through March 2013; A case series study

Reza Boostani (1), Behnaz Khazaee (1)
1. Mashhad, Iran

Objectives: Only few studies have been performed on the epidemiology and clinical aspects of ALS in the Middle East. This study aims at such, in northeastern Iran.

Methods: Demographic and clinical data of ALS patients in Khorasan province (pre-2004) were gathered and analyzed. Results: Of 71 patients, 59 fulfilled El Escorial Criteria and entered the study with a significant male preponderance (ratio: 1.8- p: 0.027). Personal and medical histories, electromyography reports (EMGs), and initial and final clinical presentations were gathered in detail. Considering the mean age of onset (47.7 years) for dividing the patients into two groups, there was no significant difference between them regarding sex, onset region and UMN/LMN pattern of onset whereas there was a significant one in disease duration of deceased patients (as a surrogate value for survival) (p:.042). Disease duration of deceased patients significantly correlated with symptom onset-diagnosis interval (p:.009- r:.670) and symptom onset-tracheostomy interval (p:.025- r:.868).

Conclusion: Mean age in our study was less than most previous studies; however, the clinical pattern was similar. Disease duration of deceased patients correlated with age at onset, symptom-diagnosis interval and symptom-tracheostomy interval.

ALS and other motor neuron diseases (except SMA) #2310

**P02-20** Sppg11 knockout mouse, a model of slowly progressive Amyotrophic Lateral Sclerosis

Julien Branchu (1), Laura Sourd (1), Alexandre Corringer (1), Esteves Typhaine (1), Raphaël Matusiak (1), Maxime Boutry (1), Khalid Hamid El Hachimi (1), Magali Dumont (1), Alexis Brice (1), Giovanni Stevanin (1), Frédéric Darios (1)
1. Inserm U1127, CNRS UMR7225, UPMC UMR_S975, Institut du Cerveau et de la Moelle épinière, Paris, France

Motor neuron diseases regroup a variety of neurological disorders with overlapping phenotypes. Among these diseases, Amyotrophic Lateral Sclerosis (ALS) is the most frequent and is characterized by spasticity, muscle weakness and wasting. The symptoms generally progress rapidly leading to death within 3 to 5 years due to the loss of both upper and lower motor neurons. Hereditary Spastic Paraplegias (HSP) constitute the second most frequent group of motor neuron diseases characterized by progressive bilateral weakness, spasticity and loss of vibratory sense in the lower limbs. These symptoms are mainly due to the degeneration of axons of the upper motor neurons of the corticospinal tract. The clinical overlap between these pathologies is supported by the mutations found in the SPG11 gene, which are the main causes of autosomal recessive HSP and are also responsible for slowly progressive autosomal recessive ALS with juvenile-onset.
SPG11 encodes a 2,443 amino acid protein named spatacsin. Regardless of the associated phenotype, the vast majority of mutations identified in SPG11 patients are nonsense or frameshift mutations, which are predicted to lead to spatacsin loss of function. To investigate how the loss of spatacsin function leads to motor neuron neurodegeneration, we generated and characterized a Spg11 knockout mouse model.

The Spg11 knockout mice showed progressive gait impairment, coordination problems and motor dysfunction that started early and worsened with time. Furthermore, all the Spg11 knockout mice exhibited muscle strength loss and half of them developed lower limb spasticity and walk with stiff legs. These behavioral deficits were associated with progressive brain atrophy with loss of neurons in the primary motor cortex and cerebellum. We also observed global spinal cord atrophy with loss of the large surface motor neurons and accumulation of dystrophic axons in the corticospinal tract. Examination of the motor neuron alterations showed that the degeneration was preceded by an accumulation of autofluorescent lipid material in lysosomal structures, which were also found in the brain of SPG11 patients.

In conclusion, the Spg11 knockout mouse recapitulates most of the clinical hallmarks observed in SPG11 patients and is a good model for slowly progressive ALS. This model will help to decipher the role of spatacsin and the physiopathology of these disabling diseases.

**Amyotrophic Lateral Sclerosis, Hereditary Spastic Paraplegia, neurodegenerative disease, animal model**

**ALS and other motor neuron diseases (except SMA)- #2641**

**P02- 21- Pathological role of amyloid peptide in Amyotrophic lateral sclerosis (ALS), glutamate and glucose involvement.**

Maud Combes (1), Philippe Poindrond (1), Noelle Callizot (1)

1. Pharmacology department, Neuro-Sys, Gardanne, France

In amyotrophic lateral sclerosis (ALS), the progressive loss of motor neurons is accompanied by extensive muscle denervation, resulting in paralysis and ultimately death. Disturbances in glutamate homeostasis, which lead to toxic accumulation of this excitatory neurotransmitter in the synaptic cleft, are observed in several neuropathologies notably in ALS. It has been recently shown that neuromuscular junction (NMJ) loss and motor neuron degeneration are substantially reduced in SOD1G93A mice when APP is genetically ablated. All together, these results indicate that endogenous APP and Amyloid beta peptide 1-42 (Aβ1-42) may contribute to ALS pathology in humans (Bryson et al., 2012). Aβ1-42 accumulation is observed in motor neurons and spinal cords of sporadic and familial cases of amyotrophic lateral sclerosis (ALS). In previous results, we used the coculture nerve/muscle cocultures as a black box and the input signals to the system are the different treatments applied to it and the output, the NMJ structure, and the length of the neurite network. We showed that glutamate and Aβ act in an interlinked manner, mutually influencing their release. We showed that NMJs are highly sensitive to Aβ peptide, that the toxic pathway involves glutamate and NMDAR (Combes et al., 2014). In light of these results, we wanted to better understand how the toxicity occurred on each cellular protagonists. To achieve this aim, we studied separately the toxicity of Aβ and glutamate and interlinks on each isolated cellular type (motor neurons and human myotubes). We showed that motor neurons were highly sensitive to Aβ and that their death involved a large release of glutamate (as already showed in cortical or hippocampal neurons). Additionally, myotubes were highly sensitive to Aβ; we showed that glutamate was not involved in this process. By contrast, glucose uptake, as well as Glucose transporter (GLUT) levels, were largely modified in presence of peptide suggesting its pathological role in skeletal muscle metabolism. From these results, Aβ may have important implication in some neuromuscular diseases (e.g., ALS) and offer new therapeutic avenues.

**ALS, Amyloid peptide, NMJ, myotube, motor neurons**

**P02- 22- AAV10-mediated exon-skipping of hSOD1 pre-mRNA rescues SOD1-linked ALS mice**

Maria Grazia Biferi (1), Mathilde Cohen-Tannoudji (1), Ambra Cappelletto (1), Marianne Roda (1), Benoit Giroux (1), Stephanie Astord (1), Thibaut Marais (1), Arnaud Ferry (2), Martine Barkats (1)

1. CNS Gene Transfer & Biotherapy of Motor Neuron Diseases, Centre de Recherche en Myologie. UMRS 974 UPMC-INSERM-CNRS FRE 3617-AIM, Paris, France
2. Myotonic Dystrophy, Physiopathology & Biotherapy, Centre de Recherche en Myologie. UMRS 974 UPMC-INSERM-CNRS FRE 3617-AIM, Paris, France

Amyotrophic Lateral Sclerosis (ALS) is an incurable adult onset motor neuron (MN) disorder characterized by upper and lower MN death, muscle atrophy, paralysis and premature death.

ALS is epidemiologically classified into sporadic (90%) and familial (10%) forms (fALS). About 20% of fALS are related to mutations in the Superoxide Dismutase 1 (SOD1) gene (Rosen et al., 1993). Mutant SOD1 has been found to acquire unknown neurotoxic properties (toxic gain of function) responsible for the pathogenic mechanism of the disease. Indeed, transgenic mice overexpressing mutant forms of the human SOD1 gene (i.e. hSOD1G93A mice) recapitulate most ALS pathological features and are widely used in preclinical studies (Gurney et al., 1995).

Suppression of the mutant SOD1 protein in affected tissues of SOD1-linked ALS patients therefore represents a promising therapeutic approach. Previous proofs of concept of this strategy have been obtained in ALS rodent models using antisense oligonucleotides (Smith et al., 2006) or Adeno Associated Virus vector (AAV)-induced RNA interference, although the rescue was still incomplete (Fouast et al., 2013; Wang et al., 2013).

We developed a new SOD1 silencing strategy by inducing splicing redirection of hSOD1 pre-mRNA (exon-skipping) using ESE-targeting antisense sequences (AS-hSOD1). This generated a premature stop codon in the transcript, thereby inducing its
degradation by the non-sense mediated decay (NMD). The AS-hSOD1 were co-delivered to both the central nervous system and the peripheral organs by combining intravenous (IV) and intracerebroventricular (ICV) injections of AAV10 vectors in newborn (P1) and adult (P50) SOD1G93A mice. AAV10-AS-hSOD1 delivery mediated efficient hSOD1 exon skipping in the spinal cord, providing high reduction of hSOD1 mRNA and protein levels (75% and 70% reduction, respectively, compared to control AAV10 injected mice).

SOD1G93A mice were dramatically rescued, with a mean increase in life expectancy of 92% and 58%, in mice injected either at birth or at 50-days of age, respectively, and a delay in disease onset of 95 days and 63 days, respectively, compared to untreated mice. AAV10-AS-hSOD1 delivery also prevented the body weight loss phenotype and preserved motor functions as well as skeletal muscle force.

This innovative and highly promising gene therapy strategy might be clinically valuable for delaying disease onset, increasing life expectancy, and improving the quality of life of SOD1-linked ALS patients.

**Gene therapy, ALS, exon-skipping**

**ALS and other motor neuron diseases (except SMA)- #2979**

P02-23- Combinatorial effects of neurotrophic factors on ALS- and SMA-relevant motor neurons isolated by high speed FACS

Sébastien Schaller (1), Dorothée Buttigieg (2), Arnaud Jacquier (2), David Gentien (3), Pierre De La Grange (4), Marc Barad (5), Georg Haase (2)

1. INT, Marseille, France
2. INT, CNRS, Marseille, France
3. Institut Curie, Paris, France
4. iPEPS-ICM, Genosplice SA, Paris, France
5. CML, Marseille, France

Several neurotrophic factors have demonstrated considerable therapeutic benefit in the motor neuron diseases amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), but the optimal factors or factor combinations remain to be identified. Using a novel high speed FACS (fluorescent-activated cell sorting) technique for the efficient isolation of pure viable mouse motor neurons, we assayed the survival effects of 12 neurotrophic factors in 66 combinations. Our data demonstrate potent, strictly additive, effects of hepatocyte growth factor (HGF), ciliary neurotrophic factor (CNTF) and artemin (ARTN) on lumbar motor neurons. We show that these additive effects are due to the existence of three distinct motor neuron subsets which specifically depend on HGF->c-Met, CNTF->Lifrb/gp130 or ARTN->GFRa3/Ret/Syndecan-3 survival signaling. The three responsive motor neuron subsets display distinct transcriptomic profiles in line with their neurotrophic factor dependence, are positioned in distinct spinal cord regions, innervate different target muscles in limb, abdomen and pelvis, and differ in their reported vulnerability to the degenerative process in ALS and SMA.

The remarkable dependence of individual motor neuron pools on specific neurotrophic factors nicely extends the neurotrophic theory and provides a new logic for the combined gene-based delivery of neurotrophic factors in preclinical ALS and SMA trials.

**motor neuron degeneration, motor neuron disease, ALS, SMA, differential vulnerability, neurotrophic factor, fluorescent-activated cell sorting, flow cytometry, gene expression profiling**

**ALS and other motor neuron diseases (except SMA)- #2983**

P02-24- Modeling C9Orf72-linked ALS in human iPSc motor neurons. Perspectives of a novel FACS-based approach.

Dorothée Buttigieg (1), Diana Toli (2), Stéphane Blanchard (2), Thomas Lemonnier (2), Boris Lamotte d'Incamps (3), Sarah Bellouze (4), Gilbert Baillat (4), Delphine Bohl (2), Georg Haase (1)

1. INT, CNRS, Marseille, France
2. Institut Pasteur, Paris, France
3. CNRS UMR 8119, Paris, France
4. INT, Marseille, France

Human motor neurons generated from induced pluripotent stem cells (iPSc) offer exciting new possibilities for disease modeling and drug testing in ALS such as ALS caused by mutations in the C9Orf72 gene. In standard iPSc-derived cultures however, cell body clustering and extensive axon criss-crossing often obscures the degeneration of motor neuron cell bodies and axons which are the two major phenotypic alterations seen in ALS. Furthermore, both variable motor neuron yield and presence of unwanted cell types limit the precision of molecular analyses.
Here, we succeeded to isolate 100% pure and standardized human motor neurons by a novel FACS double selection protocol based on the p75NTR surface epitope and an HB9::RFP lentivirus reporter. We demonstrate that the p75NTR/HB9::RFP motor neurons survive and grow well without forming clusters or entangled axons, are electrically excitable, contain ALS-relevant lateral (LMC) motor neuron subtypes and form functional connections with co-cultured myotubes. Importantly, they undergo rapid and massive cell death and axon degeneration in response to mutant SOD1 astrocytic conditioned media.

We conclude that FACS-isolated pure human iPSc-derived motor neurons allow robust modeling of toxicity induced by ALS-linked SOD1 mutations. We anticipate that they also hold considerable promise for unraveling the phenotypic defects and molecular signatures triggered by G4C2 hexanucleotide repeat expansions in the C9Orf72 gene.

induced pluripotent stem cells. iPSc. Fluorescent-activated cell sorting. Flow cytometry, p75, HB9, cell death, axon degeneration, mutant SOD1.

ALS and other motor neuron diseases (except SMA)- #2999
P02-25- Microtubule disruption as a common mechanism behind pathological Golgi fragmentation in ALS
Sarah Bellouze (1), Gilbert Baillat (1), Dorothée Buttigieg (2), Pierre De La Grange (3), Catherine Rabouille (4), Georg Haase (2)

1. INT, Marseille, France
2. INT, CNRS, Marseille, France
3. iPEPS-ICM, Genosplice SA, Paris, France
4. Hubrecht Institute of the KNAW & UMC Utrecht, Dept of Cell Biology, UMC Utrecht, Utrecht, Pays-Bas

Pathological Golgi fragmentation represents a constant pre-clinical feature of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) but its molecular mechanisms remain unclear.

Here, we show that the severe Golgi fragmentation in transgenic SOD1G85R and SOD1G93A ALS mouse motor neurons is due to defective polymerization of Golgi-derived microtubules rather than to caspase-mediated cleavage of Golgi proteins. The microtubule defects are associated with loss of the COPI coat subunit b-COP, cytoplasmic dispersion of the Golgi tether GM130, and strong accumulation of the Golgi SNAREs GS15 and GS28. Data mining, transcriptomic and protein analyses demonstrate that the microtubule-destabilizing proteins Stathmins 1 and 2 are up-regulated in mutant SOD1 motor neurons at the animal's birth and then progressively increase. Remarkably, mutant SOD1-triggered Golgi fragmentation and Golgi SNARE accumulation is recapitulated by Stathmin 1/2 overexpression and completely inhibited by RNAi-mediated Stathmin 1/2 knockdown.

We conclude that Stathmin-triggered microtubule destabilization causes defective vesicle trafficking and Golgi fragmentation in mutant SOD1-linked ALS, and propose microtubule disruption as a common mechanism mediating Golgi fragmentation in ALS, spinal muscular atrophy and related motor neuron diseases.

Amyotrophic Lateral Sclerosis, Motor Neuron, Golgi fragmentation, Superoxide dismutase 1 (SOD1), microtubule, COPI, Golgi SNARE.

ALS and other motor neuron diseases (except SMA)- #3251
P02-26- Upregulation of CRMP4 in a mouse model of Amyotrophic Lateral Sclerosis (ALS): evaluation of the cellular mechanisms and of the potential as a therapeutic target
nathalie bernard-marissal (1)
1. Lausanne, Suisse

ALS is a devastating adult-motor neuron disease characterized by the selective death of both upper and lower motoneurons (MNs). Observations made both on the ALS SOD1G93A transgenic mice, and in human ALS tissues, has revealed that MN degeneration occurs via a dying back process initiated with the disconnection of motor axons from their target muscles a long time before symptoms appearance (1). Importantly, not all MNs are equally affected during the disease: the large Vulnerable MNs innervating the Fast-twitch muscle fibers degenerate before onset of clinical symptoms, whereas the small Resistant MNs innervating slow-twitch muscle fibers degenerate only at a later stage of the disease (1). We recently demonstrated that the silencing of SOD1 expression specifically in astrocytes using AAV9-gfaABC1D-miRSOD1 prevents late muscle denervation, and extends the survival of SOD1G93A mice, indicating that astrocytes may act on the regulation of critical factors involved in the degeneration of the resistant MN pool (2). In this context, we have previously shown that Collapsin-Response Mediator Protein 4 (CRMP4) is specifically upregulated in SOD1G93A-expressing MNs following treatment with nitric oxide, a signaling molecule produced by reactive astrocytes (3), and in vivo in a subset of MNs at onset of the disease.
Our project is to determine the potential contribution of diseased astrocytes in the upregulation of CRMP4 in resistant MNs in order to develop therapeutic approaches based on CRMP4 downregulation on SOD1G93A mice.

By using an astrocyte-motoneuron coculture system, we observed a significant increase in CRMP4 specifically in SOD1G93A MNs plated on SOD1G93A astrocytes or treated with culture medium conditioned by SOD1G93A astrocytes. In vivo, the silencing of mutated SOD1 expression specifically in astrocytes, abolished the increase in CRMP4 level previously observed in SOD1G93A MNs at the onset of symptoms. Using the immunodetection of Matrix Metallopeptidase 9 (MMP9) and retrograde labeling experiments, we demonstrated that the increase in CRMP4 expression occurred mainly in the RES populations of SOD1G93A MNs.

All these results demonstrate for the first time the cooperation between glial cells and motoneurons in the selective degeneration of the resistant motoneurons, and may encourage the development of new strategies based on CRMP4 reduction for ALS.

(1) Saxena S, Nat Neurosci , 2009
(2) Dirren E, Ann Clin Transl Neurol, 2015
(3) Duplan L., J. Neurosci, 2010

crmp4, astrocytes, motoneuron degeneration

P03- Animal models- N° 27 to N° 49

Animal models- #2457

P03- 27- Dystrophin deficient rats: A robust animal model for duchenne muscular dystrophy studies

Aude LAFOUX (1), Thibaut LARCHER (2), Laurent TESSON (3), Severine REMY (3), Virginie THEPENIER (3), Caroline Le GUINIER (4), Virginie FRANCOIS (4), Lydie GUIGAND (2), Gilles TOUMANIANTZ (1), Anne De Clan (5), Charlotte BOIX (5), Jean-Baptiste RENAUD (5), Yan CHEREL (2), Carine GIOVANNANGELI (5), Jean-Paul CONCORDET (5), Ignacio ANEGON (3), Corinne HUCHET (1)

1. INSERM U1087, Université Nantes, Nantes, France
2. INRA UMR 703, ONIRIS, Nantes, France
3. INSERM U1064, Université Nantes, Nantes, France
4. Atlantic Gene Therapies, INSERM U1089, Université Nantes, Nantes, France
5. INSERM U1154, Museum National Histoire Naturelle, Paris, France

Duchenne Muscular Dystrophy (DMD) is a severe muscle-wasting disorder caused by mutations in the dystrophin gene, without curative treatment yet available. For pre-clinical evaluation of therapeutic approaches, few animal models are available. Large animal models of DMD such as dogs or pigs are expensive, difficult to handle and show important clinical heterogeneity, while mdx mice exhibit only limited chronic muscular lesions and muscle weakness. A rat model could represent a useful alternative since rats are small animals but 10 times bigger than mice and could better mimic the human disease.

A line of Dmd mutated-rats (Dmdmdx) was generated using TALENs. Animals showed undetectable levels of dystrophin by western-blot and less than 5 % of dystrophin positive fibers by immunohistochemistry in muscles analyzed. The results showed that Dmdmdx rats have significantly reduced body weight from 4 weeks. At 3 months, limb and diaphragm muscles displayed intense necrosis and re-organization. At 7 and 12 months, these muscles showed severe fibrosis and adipose tissue infiltration. From 6 weeks, Dmdmdx rats showed significant reduction in muscle strength associated with muscular fatigue and a decrease in spontaneous motor activity. Concerning the heart, echocardiography showed significant concentric remodeling and alteration of diastolic function at 3 months. Subsequently, the heart morphology evolved into a dilated cardiomyopathy with necrotic and fibrotic tissue. A long-term study showed that life span was reduced in Dmdmdx rats. Furthermore, cardiac insufficiency or dilated cardiomyopathy were frequently the direct cause of death of these Dmdmdx rats.

In conclusion, Dmdmdx rats represent a very promising small animal model that can be used now for pre-clinical evaluation of therapeutic approaches of DMD, in particular for testing effects on disease progression and cardiac anomalies that were difficult to assess using the current DMD animal models.

animal model, muscular dystrophy, skeletal muscle, heart, TALENs

Animal models- #2526

P03- 28- Characterization of a DmdEGFP reporter mouse

Mina V. PETKOVA (1), Susanne Morales-Gonzales (1), Esther Gill (1), Franziska Seifert (1), Josefine Radke (2), Werner Stenzel (2), Luis Garcia (3), Helge Anmthor (3), Markus Schuette (1)

1. NeuroCure Clinical Research Center, Charité?Universitätsmedizin, Berlin, Allemagne
2. Department of Neuropathology, Charité?Universitätsmedizin, Berlin, Allemagne
3. INSERM U1179 and LIA BAHN Centre scientifique de Monaco, Montigny-le Bretonneux, Université de Versailles St-Quentin, Saint-Quentin-en-Yvelines, France

We generated a novel DmdEGFP reporter mouse, in which an EGFP coding sequence was inserted behind exon 79 of the Dmd gene. This exon is known to be present in most dystrophin isoforms and splice variants, as well as in revertant dystrophin. To date no dystrophin reporter mice exist and imaging is only possible by indirect antibody-mediated staining ex-vivo. We characterized this mouse model and found by Western blot analysis normal dystrophin expression levels in limb muscles, diaphragm, heart, brain, and retina. We found high native EGFP fluorescence at all expected sites of dystrophin expression. Skeletal muscles showed normal histology as well as sarc membranesubsarcolemmal localization of dystrophin-EGFP and of