PW 6:
SMA and ALS – Molecular pathogenesis and treatment

PW6-065	**SMN IS REQUIRED FOR FUNCTIONAL REORGANIZATION OF METHYLATED NUCLEOLAR PROTEINS DURING SKELETAL MUSCLE DIFFERENTIATION** PAQUETTE B[1], CÔTÉ J[1] (1) University of Ottawa, Ottawa, CANADA.
To contact the author:: jcote@uottawa.ca.	Deletions or loss-of-function mutations in the Survival of Motor Neurons 1 (Smn1) gene in humans is responsible for Spinal Muscular Atrophy (SMA), one of the leading genetic causes of infant mortality. The pathological hallmarks of this disease include the degeneration of lower motor neurons in the anterior horn of the spinal cord, weakness, paralysis and atrophy of the associated skeletal muscles and eventually of the entire trunk, often times causing respiratory failure and early death during disease progression. Although current knowledge support the notion that this disease arises from the massive motoneurons loss early in development, mounting evidence suggest that intrinsic muscle defects may also contribute to the pathology. Arginine methylation is also known to be important for normal skeletal muscle differentiation. Since SMN can serve as an adaptor module for arginine methylated proteins, we speculated that SMN will perform its function in skeletal muscles by promoting methyl-dependent interactions. This study has documented a dynamic profile of proteins containing methylated arginines during myoblast differentiation, and that this dynamic profile is dependent on the presence of functional levels of SMN. Furthermore, we have identified Fibrillarin, a snoRNP component, as one of the methylated proteins that are misregulated in the absence of SMN. Finally, we have also showed that the profile of arginine methylated proteins differs between skeletal muscles from wild type and SMA model mice. Taken together, these results represent a novel defect in SMA and provide evidence for the importance of skeletal muscle tissue in this disease.

PW6-066	**A NOVEL APPROACH TO SPINAL MUSCULAR ATROPHY THROUGH MODULATION OF PGC-1ALPHA.** ARNOLD AS[1], HANDSCHIN C[1] (1) Zürich Center for Integrative Human Physiology (ZIHP), Zürich, SWITZERLAND.
To contact the author:: a.arnold@access.uzh.ch .	Spinal muscular atrophy (SMA) is characterized by a mutation in the survival motor neuron (SMN) gene, leading to a deficit of expression of the SMN protein, a ubiquitously expressed protein which exact function is unknown. It results in motoneuron degeneration and muscle atrophy. For a long time, SMA was considered as a motor neuron disease only, but recent findings involve the muscle in the development of the pathology, as well. We showed that a key regulator of muscle plasticity, the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1), is able to protect muscle from disuse-induced atrophy and to regulate the expression of post-synaptic genes in the neuromuscular junction (NMJ). Many properties of PGC-1 . as well as its beneficial effects on dystrophic phenotype in mdx mice, suggest that it might be a potential therapeutic target in SMA.For this study, we have three different transgenic mice: SMA models (SMA I and SMA III) and a PGC-1 global KO mouse. The expression of PGC-1 , SMN and other RNA metabolism genes assessed by real-time PCR, and the expression of specific markers for the NMJ (acetylcholine receptors, neurofilaments, synaptophysin…) observed by immunohistochemistry showed differences between those models. These experiments give insights into the possible mechanistic links between PGC-1 and SMN at the muscle and the NMJ levels. We have at our disposal different constructs allowing us to over-or under-express either SMN or PGC-1 in primary muscle cells coming from our models. By using different combinations of those constructs, followed by the innervations of the cells with rat spinal cords explants, we can observe the behavior of the nerve-muscle cocultures and highlight the possible beneficial effect of PGC-1 on the SMA phenotype. Those preliminary results will open the way for a brand new field in the treatment of SMA, a devastating disease for which therapeutic breakthrough are desperately needed.

PW6-067	**ANTISENSE OLIGOMER (AO)-INDUCED EXON INCLUSION IN SPINAL MUSCULAR ATROPHY (SMA) FIBROBLASTS** MITRPANT C[1], FLETCHER S[1], WILTON S[1] (1) Australian Neuromuscular Research Institute (ANRI), Perth, AUSTRALIA.
	Spinal Muscular Atrophy (SMA) is a group of autosomal recessive neuromuscular diseases, characterized by progressive symmetrical muscle weakness with muscle atrophy. Deletion of the Survival Motor Neuron1 (SMN1) gene causes degeneration of anterior horn cells of the spinal cord. Although SMN2, a centromeric copy of SMN1, is present, a single base difference in exon 7 of SMN2 leads to exclusion of exon 7 which, in turn, disrupts the reading frame and dramatically reduces the amount of functional protein. Antisense Oligomers (AOs) have been widely used in gene inactivation applications. Oligomer action is determined by the base composition, chemistry, and nature of the backbone, thereby allowing modification of gene expression at different stages of pre-mRNA processing. AOs can modify pre-mRNA splicing by masking exonic sequence enhancer motifs (ESEs), involved in exon definition and splicing. ESEs, which are well-defined as positive regulatory elements appear crucial to splicing, as many mutations disrupting ESEs cause exon skipping. On the contrary, splice silencing motifs are less well-characterized. To induce exon inclusion, we designed an array of AOs to identify and suppress silencing splice motifs. The AOs were evaluated for exon inclusion efficiency in fibroblast cells derived from a SMA patient. Combinations of AOs were employed to assess interplay between different targeted motifs involving in pre-mRNA splicing. Several potential silencing motifs were identified as determined by RNA and protein studies. This preliminary data will be used to design additional AOs for efficient exon 7 inclusion.

PW6-068	**EXPRESSION OF RNA-BINDING PROTEINS THAT CONTROL RNA STABILITY IN ATROPHYING OR HYPERTROPHYING SKELETAL MUSCLE CELLS.** CHOPARD A[1], BRONICKI L[2], HILLOCK S[2], LUNDE J[2], JASMIN BJ[2] (1) Université de Nice Sophia Antipolis, and Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Road, K1H 8M5, Ottawa, CANADA. (2) Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Road, K1H 8M5, Ottawa, CANADA.
To contact the author:: angele.chopard@gmail.com.	Several transcriptional mechanisms are known to be involved in the atrophic-hypertrophic response of skeletal muscle. However, converging lines of evidence have led us to hypothesize that post-transcriptional events, operating at the level of mRNA stability, are also likely contributing. We thus initiated a series of studies to determine the role of post-transcriptional mechanisms in the adaptive response of muscle cells to stimuli that cause profound changes in their size. Given the key role of AU-rich elements (AREs) located in the 3'UTR of multiple mRNAs in controlling their stability, we initially focused on the contribution of RNA-binding proteins (RBPs) known to interact with this cis-element. Specifically, we examined expression of HuR (ELAV1), AUF1 (HNRPD), and TTP (ZFP36) at the protein and mRNA levels, in muscle cells challenged with atrophic or hypertrophic stimuli. Using short-term denervation of mouse hindlimb muscles, as well as an *in vitro* model of atrophy (via dexamethasone treatment of C2C12 cells), we observed a rapid, substantial and consistent increase in the expression of destabilizing (AUF1 and TTP) and stabilizing (HuR) factors in atrophying muscle cells. Functional overload of the mouse plantaris muscle and *in vitro* treatment of C2C12 myotubes with IGF-1 (to promote muscle growth), both resulted in a decrease in expression of AUF1 and HuR, as compared to control cells. Together, these data highlight the contribution of post-transcriptional events in controlling the adaptation of muscle to various conditions. Since these factors bind to AREs found in multiple important mRNAs, our results identify novel molecular mechanisms that likely play a key role in mediating both the atrophic and hypertrophic response of muscle cells. Furthermore, our findings provide additional targets that may be useful for developing therapeutics aimed at countering muscle atrophy or degeneration, and/or at promoting muscle growth. Funded by AFM, CSA, CNES, MDA and CIHR.

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<tr><td>PW6-069</td><td><u>PROTEOMICS-BASED IDENTIFICATION OF PROTEINS ASSOCIATED WITH THE SPINAL MUSCULAR ATROPHY GENE PRODUCT, SMN</u>
RENVOISÉ B[1], HAMAÏ S[1], MONTAGNE JJ[2], GENDRON MC[3], LEFEBVRE S[1]
(1) Laboratoire de Biologie Cellulaire des Membranes, Institut Jacques Monod, UMR7592 CNRS/Univ. Paris 6 et 7, Paris, FRANCE. (2) Protéomique, Institut Jacques Monod, UMR7592 CNRS/Univ. Paris 6 et 7, Paris, FRANCE. (3) Cytométrie en flux, Institut Jacques Monod, UMR7592 CNRS/Univ. Paris 6 et 7, Paris, FRANCE.</td></tr>
<tr><td>To contact the author::
lefebvre@ijm.jussieu.fr.</td><td>Spinal muscular atrophy (SMA) is caused by mutations of Survival Motor Neuron 1 (SMN1) gene, which lead to reduction of SMN protein levels. The ubiquitous SMN complex participates to the biogenesis of spliceosomal core snRNPs and together are enriched into nuclear gems/Cajal Bodies (CBs). There is a tight correlation between the number of gems/CBs and the severity of SMA disease. Moreover, gems/CBs from SMA-derived fibroblast cells show no detectable accumulation of snRNPs. The mechanism by which the SMN complex and snRNPs are associated/dissociated from gems/CBs remains elusive. It has been shown in recent studies that phosphorylation might regulate the function and localization of the SMN complex. Here, protein complexes were immunoprecipitated from COS cells stably expressing eGFP-tagged SMN mutants using anti-GFP antibodies. Silver staining of the coeluted proteins separated by SDS-PAGE revealed a band of about 42-kDa that was analysed by mass spectroscopy. A nuclear serine/threonine protein phosphatase was identified on the basis of 32 peptides covering 68% of its sequence. This enzyme might be critical for the biochemical properties of the SMN protein. We have characterized the association of this protein phosphastase with the SMN protein using immunoprecipitation experiments, siRNA-mediated depletion, 2D-PAGE and immunofluorescence analyses. These findings will be reported. Our proteomics-based strategy is to decipher the molecular functions of the SMN protein in gems/CBs, to improve our understanding of the pathological processes and hopefully, help clinical pratice.</td></tr>
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PW6-070	**SCREENING OF BIOACTIVE MOLECULES TOWARDS THE DISCOVERY OF PHARMACOLOGICAL THERAPEUTICS FOR SPINAL MUSCULAR ATROPHY** COLASSE S[1], KHOOBARRY K[1], RENVOISÉ B[1], LEFEBVRE S[1] (1) Laboratoire de Biologie Cellulaire des Membranes, Institut Jacques Monod, UMR7592 CNRS/Univ. Paris 6 et 7, Paris, FRANCE.
To contact the author:: lefebvre@ijm.jussieu.fr.	Infantile spinal muscular atrophy (SMA) is an inherited disorder characterized by the degeneration of spinal motor neurons and progressive muscular atrophy. There is no cure for SMA. The SMA disease is caused by mutations of the survival motor neuron 1 (SMN1) gene. SMN1 gene is duplicated and all patients carry the copy gene SMN2 that modulates the phenotype by a reduced expression of the SMN protein. This ubiquitously expressed protein plays a role in RNA processing, including the assembly and localization of spliceosomal core snRNPs. SMN protein localizes in the cytoplasm and is enriched with snRNPs into nuclear gems/Cajal Bodies (CBs). There is a tight correlation between the levels of SMN protein, the number of gems/CBs and the severity of SMA disease. Moreover, gems/CBs from SMA-derived fibroblast cells show no detectable

PW6-071	**ANALYZING SPINAL MOTONEURON DIVERSITY BY FACS** BARAD M[2], JACQUIER A[1], BOHL D[3], BLANCHARD S[3], BUHLER E[1], MEDINA I[4], HAASE G[1] (1) Equipe Avenir, INSERM-Université de la Méditerranée, Marseille, FRANCE. (2) CIML, INSERM-CNRS-Université de la Méditerranée, Marseille, FRANCE. (3) Institut Pasteur, INSERM U622, Paris, FRANCE. (4) INMED, INSERM-Université de la Méditerranée, Marseille, FRANCE.
To contact the author:: haase@ibdml.univ-mrs.fr.	Spinal motoneurons display a remarkable diversity in cell body diameter, axonal trajectories and motor unit size. To study intrinsic and extrinsic factors determining this diversity we developed novel flow cytometry and FACS-based techniques. Here, we FACS-isolated mouse E12.5 axial and hindlimb motoneurons, monitored their gene expression profile and compared their electrical and morphological properties in culture. Hindlimb motor neurons had a high whole cell capacitance indicating a large soma and were responsive to HGF (hepatocyte growth factor), a neurotrophic factor produced in the limb mesenchyme. Hindlimb motoneurons grew long axons with few mostly terminal branches. In contrast, axial motor neurons had a low whole cell capacitance, were unresponsive to HGF and grew short axons with numerous proximal branches. Hb9 over-expression in limb motor neurons forced them to adopt an axonal morphology reminiscent of axial motor neurons suggesting a direct role of Hb9 in axon growth and branching. We conclude that the distinct morphologies of limb and axial motor neurons reflect their respective roles in fine versus bulk movement and their reported differences in motor unit size.

PW6-072	**DISTRIBUTION OF RIBONUCLEOPROTEIN COMPLEXES IN CAJAL BODIES FROM SMA TYPE I-DERIVED FIBROBLAST CELLS** RENVOISÉ B[1], LEFEBVRE S[1] (1) Laboratoire de Biologie Cellulaire des Membranes, Institut Jacques Monod, UMR7592 CNRS/Univ. Paris 6 et 7, Paris, FRANCE.
To contact the author:: lefebvre@ijm.jussieu.fr.	Spinal Muscular Atrophy (SMA) is a common neurodegenerative disease caused by reduced levels of the Survival Motor Neuron (SMN) protein. SMN is part of a large ubiquitous protein complex that concentrates in nuclear gems/Cajal bodies (CBs) and participates in the biogenesis of various ribonucleoproteins, including the spliceosomal UsnRNPs (U-rich small nuclear RiboNucleoProteins) and ribosomal processing snoRNPs (small nucleolar RNPs). The newly synthesized UsnRNPs are accumulated and modified in the maturation platform gems/CBs and then are redirected to the splicing messenger RNA sites and in the speekles. It has been also shown that snoRNPs are transported through CBs for modifications and then addressed to nucleoli. The numbers of gems/CBs are in close correlation with the severity of SMA disease. We previously showed that UsnRNPs are not detectable in gems/CBs from immortalized SMA type I-derived fibroblast cells, indicating that UsnRNP maturation pathway is probably inhibited in SMA disease. Here, we have pursued the characterization of residual gems/CBs in immortalized SMA fibroblast cells. We have tested factors transiently accumulating in gems/CBs that are involved in the assembly, maturation and localization of snoRNPs. We have examined those factors using immunofluorescence analyses, transient expression of SMA mutants SMNDex7, 472D5 and E134K and RNA-interference knockdown in HeLa cells. These results will be presented. They should allow a better understanding of the mechanisms underlying the accumulation in *gems*/CBs and of the functional defect and pathological processes involved in the SMA disease.

PW6-073	**NEURODEVELOPMENTAL ABNORMALITIES IN NEURAL STEM CELLS AND EMBRYOS FROM SMN DEPLETED MICE** SHAFEY D[1], LIU H[1], BOWERMAN M[1], KOTHARY R[1] (1) Ottawa Health Research Institute, Ottawa, Ontario, CANADA.

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Spinal muscular atrophy (SMA) is the most common genetic disease resulting in infant mortality. SMA manifests itself due to a severe loss of α-motor neurons and is caused by mutations or deletions of the ubiquitously expressed survival motor neuron 1 (*SMN1*) gene. However, why α-motor neurons of SMA patients are specifically affected is not clear. We have previously demonstrated that Smn-deficient PC12 cells show altered Profilin II expression and an upregulation of the RhoA/ROCK pathway accompanying defects in neuronal integrity. Here, we have investigated the morphology and differentiation of neurosphere-derived neural stem cells (NSCs) generated from the brains of a hypomorphic series of SMA mice. Neurospheres from the *Smn*$^{-/-}$*;SMN2* mice, which represent a model of very severe SMA, produced NSCs with increased proliferation during growth and differentiation. These cells produced fewer Tuj1-positive neuronal cells, which displayed morphological alterations and had fewer and shorter neurites. The decrease in Tuj1-positive cells was not due to enhanced apoptosis but was accompanied by an increase in the number of nestin-positive cells. These results give us insight into the most severe model of SMA in which SMN is nearly completely depleted, and suggest that SMN has a role in neurodevelopment as well as in neuromaintenance. Our work raises the possibility that SMN depletion impacts neurodevelopment and neuromaintenance to varying extents leading to SMA pathogenesis. We are currently investigating the impact of Smn depletion on embryonic development using the *Smn*$^{-/-}$*;SMN2* mouse as a model.

PW6-074

A NOVEL MOTONEURON DEATH PATHWAY MEDIATED BY THE TNF SUPERFAMILY LIGAND, LIGHT

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Death pathways restricted to specific neuronal classes could explain the selectivity of neuronal loss in neurodegenerative diseases, such as the loss of motoneurons in amyothrophic lateral sclerosis (ALS). We previously showed that Fas-induced death of motoneurons involves a motoneuron-specific cell death pathway, which is exacerbated in motoneurons expressing ALS-linked mutated SOD1[1-2]. Importantly, involvement of Fas death pathway in the pathogenesis has been described[3]. However, Fas death pathway may not be responsible for the loss of all motoneurons, suggesting that other death pathways might be implicated.
LIGHT is a member of the tumor necrosis factor receptor superfamily, which upon binding to the herpes virus entry mediator (HVEM) and/or the lymphotoxin- receptor (LT- R) can trigger death program in tumor cells. We show that cultured motoneurons express both LT- R and HVEM, and that soluble recombinant LIGHT induces death of about 50% of motoneurons through a non-classical caspase-dependent pathway. LIGHT-promoted death appears to be selective of motoneurons, since striatal, cortical and hippocampal neurons are resistant to LIGHT killing effect. Strikingly, we showed that LIGHT is additive to FasL, indeed both ligands, added together induce death of about 70% of motoneurons. We are currently investigating the role of LIGHT in the pathogenesis of ALS in animal models and its potential as a therapeutic target.

1. Raoul, C. *et al.* (1999). J Cell Biol 147, 1049-62.
2. Raoul, C. *et al.* (2002). Neuron 35, 1063-87.
3. Raoul, C. *et al.* (2006). Proc Natl Acad Sci USA 103, 6007-12.

PW6-075	**<u>DECREASED EXPRESSION OF AN ER CHAPERONE, CALRETICULIN, IN ALS-MODEL MICE MOTONEURONS: CAUSE OR CONSEQUENCE IN MOTONEURON DEATH?</u>** BERNARD N[1], DUPLAN L[1], MARIN P[2], RAOUL C[1], PETTMANN B[1] (1) INSERM-Avenir Team, The Mediterranean Institute of Neurobiology, INMED, Marseille, FRANCE. (2) Institut de Génomique Fonctionnelle, CNRS UMR 5203, INSERM U661, Montpellier, FRANCE.
To contact the author:: pettmann@ibdml.univ-mrs.fr.	Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving degeneration of the upper and lower motoneurons leading to progressive muscle atrophy. The best caracterized familial form of this disease is linked to mutations in the gene coding for the superoxide dismutase 1 (SOD1). We previously showed that cultured embryonic motoneurons expressing human mutant Superoxide Dismutase 1 (mSOD1) present an increased sensitivity to death induced by Nitric oxide (NO). In order to identify potential effectors in this death pathway, we realized a proteomic study which revealed a two-fold decrease in the expression of an ER chaperone/calcium storage protein, calreticulin, in mSOD1 motoneurons after NO treatment. Fluorescence intensity quantification studies confirmed a decrease of calreticulin in motoneurons expressing two different mutated SOD1 (mSOD1), but not in motoneurons overexpressing the human wildtype SOD1. We showed that the calreticulin decreased expression is cell type specific and occuring after an excitotoxic stress but not after a factor deprivation stress. Interestingly, in mSOD1mice, calreticulin expression is diminished in the lumbar and brachial but not thoracic motoneurons, at asymptomatic (60d), presymptomatic (90d) and symptomatic (112d) stages of the disease. We will now attempt to determine which role the decrease in calreticulin could play in disease progression and motoneuron death in ALS. Calreticulin is implicated in the folding of nascent proteins and also in Ca2+ storage. The accumulation of misfolded protein and/or Ca2+ depletion in ER might lead to ER stress and potentially to death. After overexpressing or silencing calreticulin in wildtype and mSOD1 motoneurons, we will study the consequences on survival, mSOD1 aggregation, Ca2+ pool changes and ER stress pathway activation. These experiments should indicate whether calreticulin could represent a good therapeutic target in ALS.

PW6-076	**ANALYSIS OF THE SWIMMING-INDUCED BENEFITS IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS.** DEFORGES S[1], BIONDI O[1], PARISET C[1], LOPES P[1], LÉCOLLE S[1], GALLIEN CL[1], CHANOINE C[1], CHARBONNIER F[1] (1) Université Paris Descartes - CNRS, Paris, FRANCE.
To contact the author:: frederic.charbonnier@univ-paris5.fr.	Amyotrophic Lateral Sclerosis (ALS) is a fatal adult-onset neurodegenerative disease that involves selective loss of motor neurons in the motor cortex, brainstem and spinal cord. The benefits of regular exercise for ALS still remain controversial. The use of different exercise paradigm in mice led to contradictory results, suggesting that exercise effects might depend on exercise protocol. We have recently designed a new swimming-based training protocol associated with high frequency and amplitude exercise. We compared the time course of muscle adaptations to the swimming- and a running-based training exercise in healthy mice. We demonstrated that the magnitude of exercise-induced muscle plasticity proved to be dependent on both the muscle type and the exercise paradigm. Importantly, we showed that, in our experimental conditions, swimming predominantly requests rapid motor units and, consequently, larger-sized lumbar motor-neurons, as indicated by a double labeling using the activity marker c-fos and a ChAT immunodetection. Since ALS is characterized by a preferential loss of fast motor units, involving thus the larger-sized motor neurons, we submitted a population of transgenic hSOD1 mice to our swimming program from 70 days of age, and compared the swimming effects with those of a running protocol. Only the swimming program resulted in a significant delay in the onset of symptoms and in a survival improvement. Furthermore, our results clearly indicated that the swimming-based training imposed on hSOD1 mice protects the motor neurons, contrary to the running-based training. These results strongly suggest that the exercise-activated neurons are selectively protected against cell death, in contrast to resting neurons. In this respect, the molecular mechanism(s) underlying exercise neuroprotection is indicative of an intrinsic modification of the activated neuron. Further identification of these mechanisms may constitute a promising therapeutic way.