

**PW 34:  
Animal models (Part 2)**

PW34-425	<p><b><u>TRANSGENIC MICE WITH THE FULL-LENGTH HUMAN DMD GENE FACILITATE STUDY OF TRANSCRIPTION REGULATION AND GENETIC THERAPIES FOR DUCHENNE MUSCULAR DYSTROPHY</u></b></p> <p>T HOEN P<sup>1</sup>, DE MEIJER E<sup>1</sup>, AARTSMA-RUS A<sup>1</sup>, VAN OMMEN G<sup>1</sup>, VAN DEUTEKOM J<sup>1</sup>, DEN DUNNEN J<sup>1</sup></p> <p>(1) Leiden University Medical Center, Department of Human Genetics, Leiden, THE NETHERLANDS.</p>
To contact the author:: p.a.c.hoen@lumc.nl.	<p>We have generated mice with an intact and functional copy of the 2.3 Mb human dystrophin gene (<i>hDMD</i>), the largest functional stretch of human DNA thus far integrated into a mouse chromosome. For this, yeast spheroplasts containing an artificial chromosome with the full-length <i>hDMD</i> gene were fused with mouse embryonic stem cells, and were subsequently injected in mouse blastocysts to produce transgenic <i>hDMD</i> mice. Human-specific PCR, Southern blotting and fluorescent in situ hybridization techniques demonstrated the intactness and stable chromosomal integration of the <i>hDMD</i> gene on mouse chromosome 5. Expression of the transgene was confirmed by RT-PCR and Western blotting. The tissue-specific expression pattern of the different <i>DMD</i> transcripts was maintained. However, the human Dp427p and Dp427m transcripts were expressed at two-fold higher levels, and human Dp427c and Dp260 transcripts at two- and four-fold lower levels than their endogenous counterparts. Ultimate functional proof of the <i>hDMD</i> transgene was obtained by crossing of <i>hDMD</i> mice with dystrophin-deficient <i>mdx</i> mice and dystrophin and utrophin-deficient <i>mdx</i> x <i>Utrn</i><sup>-/-</sup> mice. The <i>hDMD</i> transgene rescued the lethal dystrophic phenotype of the <i>mdx</i> x <i>Utrn</i><sup>-/-</sup> mice. All signs of muscular dystrophy disappeared in the rescued mice, as demonstrated by histological staining of muscle sections and gene expression profiling experiments. Currently, <i>hDMD</i> mice are extensively used for pre-clinical efficacy testing of exon skipping antisense oligonucleotides, which restore the reading frame in patients with Duchenne muscular dystrophy. In addition, the <i>hDMD</i> mouse can be used to study the influence of the genomic context on deletion and recombination frequencies, genome stability, and gene expression regulation.</p>

PW34-426	<p><b><u>QUANTITATIVE CHARACTERISATION OF DYSTROPHIC MUSCLE IN GRMD DOGS BY NMR IMAGING.</u></b>          THIBAUD JL<sup>1</sup>, BERTOLDI D<sup>2</sup>, MONNET A<sup>2</sup>, BARTHÉLÉMY I<sup>1</sup>, BLOT S<sup>1</sup>, CARLIER PG<sup>2</sup>          (1) Laboratoire de Neurobiologie ENVA, Maisons-Alfort, FRANCE. (2) Laboratoire de RMN AIM - CEA Institut de myologie, Hopital Pitié-Salpêtrière, Paris, FRANCE.</p>
To contact the author:: jlthibaud@vet-alfort.fr.	<p>The Golden Retriever Muscular Dystrophy dog lacks dystrophin and shares pathological and clinical similarities with the Duchenne patients. The model, increasingly used in pre-clinical trials, needs to be further characterized. We defined NMR imaging indices of canine dystrophic muscle. Six two-month old control and 6 GRMD dogs were examined at 4 T. Three control and 5 GRMD dogs were examined at 3 T at the age of 2, 4 and 6 months. Standard and fat-saturated T1-weighted images were acquired, followed by T2-weighted images. After Gd-DTPA injection, the time-course of muscle enhancement was monitored with fat-saturated T1-weighted imaging during 2 hours. <i>Extensor carpi radialis</i> and <i>flexor carpi ulnaris</i> were studied. Indices were calculated as follows: T2w/T1w signal ratio (SR= T2w Signal x T1w ref gain)/ T1w Signal x T2w ref gain), T1w and T2w heterogeneity (<math>H = \sqrt{(SD^2 - SD^2_{noise}/0,655)}</math>). An exponential decay was fitted to the signal decrease post Gd-DTPA injection; maximal relative enhancement (RE) and time-constant of decay were compared. A three-way analysis of variance was performed. T2w/T1w SR, H2 and RE were found significantly increased in dystrophic muscles at 4 T. These findings were confirmed at 3 T, at all ages. H1 was also found significantly increased in dystrophic muscle at 4 and 6 months at 3 T.</p> <p>These quantitative indices differentiate dystrophic from normal muscles and might be proposed as non-invasive evaluation tools of therapeutic trials.</p>

PW34-427	<p><b><u>CENTRONUCLEAR MYOPATHY: DECIPHERING PHYSIOPATHOLOGICAL MECHANISMS IN SKELETAL MUSCLES AND VISCERAL ORGANS THROUGH THE ANALYSIS OF THE PTPLA GENE FUNCTION IN LABRADOR RETRIEVERS AFFECTED BY CNM.</u></b></p> <p>MAURER M<sup>1</sup>, MARY J<sup>1</sup>, DROUGARD C<sup>1</sup>, BARTHÉLÉMY I<sup>2</sup>, BERNEX F<sup>1</sup>, GUILLAUD L<sup>1</sup>, KESSLER JL<sup>1</sup>, PELÉ M<sup>1</sup>, PANTHIER JJ<sup>1</sup>, BLOT S<sup>2</sup>, TIRET L<sup>1</sup></p> <p>(1) UMR955 INRA-ENVA, ENV Alfort, Maisons-Alfort, FRANCE. (2) Laboratoire de Neurobiologie, ENV Alfort, Maisons-Alfort, FRANCE.</p>
To contact the author:: mmaurer@vet-alfort.fr.	<p>The autosomal recessive centronuclear myopathy of the Labrador Retriever (<i>cnm</i>) is a relevant clinical and histopathological model for the human nosological group of myotubular/centronuclear myopathies. We previously showed that the disease-causing gene was <i>PTPLA</i>, encoding the Protein Tyrosine Phosphatase-Like, member A. In skeletal muscles of affected dogs, we demonstrated that the two copies of the <i>PTPLA</i><sup>aff</sup> mutated allele were prone to several splicing abnormalities, eventually resulting in the production of only 1% of the normal level of wild-type transcripts. In Labradors, the <i>cnm</i> mutation is therefore hypomorphic at the transcriptional level.</p> <p>In man, the genomic coding sequence of the orthologous <i>PTPLA</i> gene has been assessed in some affected individuals. No significant functional polymorphism could be found. Thus, although not a true molecular homologue of the human disorder, the canine <i>cnm</i> model remains highly relevant to analyze and compare the physiopathological mechanisms leading to similar diseases in both species.</p> <p>In mammals, the function of the <i>PTPLA</i> protein remains undeciphered. Using antibodies currently being fully characterized, we have initiated the description of its cellular and subcellular expression pattern in healthy and affected dogs, as well as in cell lines and mouse embryos. Two splicing variants of <i>PTPLA</i> (<i>PTPLAfl</i> and <i>PTPLAd5</i>) were exclusively expressed in different organs. <i>PTPLAfl</i> was expressed mainly in skeletal muscles and heart. In affected dogs, we found that the muscular T-tubule network and its associated proteins (DHPR, BIN1) was abnormally spread in some fibers. The excitation-contraction coupling could therefore be implicated in the physiopathological mechanisms of the disease. <i>PTPLAd5</i> was present in several smooth muscle fibers containing organs, and sub-clinical defects could be observed in some of them in healthy carriers and affected dogs. This highlighted an unexpected key role played by <i>PTPLA</i> in cellular homeostasis, yet to be investigated in man.</p>

PW34-428	<p><b><u>SYNAPSE AND AXON DEGENERATION IN THY1 YFP MICE WITH PROGRESSIVE MOTOR NEURONOPATHY</u></b>          BIELLI S<sup>2</sup>, SCHÄFER M<sup>1</sup>, BELLOUZE S<sup>1</sup>, HAASE G<sup>1</sup>          (1) Equipe Avenir, INSERM-Université de la Méditerranée, Marseille, FRANCE. (2) Trophos SA, Marseille, FRANCE.</p>
To contact the author:: haase@ibdml.univ-mrs.fr.	<p>Progressive motor neuronopathy (<i>pmn</i>) is a particularly aggressive form of motor neuron disease characterized by early loss of neuromuscular synapses and axonal dying back (Schmalbruch et al., 1991). We previously identified the genetic defect of <i>pmn</i> mice as a missense mutation in TBCE, one of five tubulin chaperones required for tubulin folding and dimerisation (Martin et al. 2002). We further demonstrated that TBCE is a Golgi-associated protein that controls axonal tubulin routing and microtubule maintenance (Schäfer et al. 2007).</p> <p>To monitor the morphological and molecular correlates of progressive motor neuronopathy we now crossbred <i>pmn</i> mice with transgenic <i>thy1</i>-YFP-reporter mice (Feng et al. 2000) and labelled whole mount nerve-muscle preparations from endstage mice for VAcHT (vesicular acetylcholine transporter), synaptic vesicle proteins and acetylcholine receptors. Axon degeneration was attested by calibre irregularities of YFP-positive axons, axonal spheroids and loss of axonal continuity. Degeneration of neuromuscular synapses was evidenced by reduced VAcHT levels in presynaptic terminals and by altered distribution of postsynaptic acetylcholine receptor clusters. These degenerative changes were most pronounced in the diaphragm and more moderate in the gluteus and superficial abdominal muscles. <i>Thy1</i> YFP mice thus represent a powerful tool to monitor temporal progression and spatial distribution of axon and synapse degeneration in mouse motor neuron disease.</p>

PW34-429	<p><b><u>MEF2C IS PRECOCIOUSLY INVOLVED IN XENOPUS LAEVIS TENDON DEVELOPMENT</u></b>  DELLA GASPERA B<sup>1</sup>, ARMAND AS<sup>1</sup>, LECOLLE S<sup>1</sup>, CHARBONNIER F<sup>1</sup>, CHANOINE C<sup>1</sup>  (1) Université Paris Descartes, CNRS, Paris, FRANCE.</p>
<p>To contact the author::  christophe.chanoine@univ-paris5.fr.</p>	<p>The myocyte enhancer factor 2 (MEF2) family of MADS (MCMI, agamous, deficiens, serum response factor)-box transcription factors has four members in vertebrates, MEF2A, -B, -C and -D. The four MEF2 genes are expressed in complex and overlapping patterns in embryonic and adult tissues. MEF2 family members have been shown to play a pivotal role in morphogenesis and myogenesis of skeletal, cardiac and smooth muscles cells differentiation and also regulates neuronal and immune cell differentiation. In mice, two unanticipated roles for MEF2C have been recently identified showing that MEF2C controls both chondrocyte hypertrophy and bone development and that the expression of MEF2C in the neural crest is required for craniofacial development.</p> <p>In this study, we describe the cloning of multiple <i>Xenopus</i> MEF2 splicing isoforms differentially expressed during development and show that the accumulation of XMEF2C mRNA was initially specifically located in forming and migrating neural crest. In the tadpole stages, XMEF2C expression was restricted to intersomitic regions and to the peripheral edges of epaxial and cranial muscle masses and was coexpressed with scleraxis, a specific marker for tendons and ligaments.</p> <p>We show that overexpression of XMEF2C inhibited myogenesis, whereas the use of an hormone-inducible MEF2C construct is able to induce scleraxis expression. Furthermore, XMEF2C and scleraxis act cooperatively to induce betaig-h3, a gene expressed in collagen-rich tissues.</p> <p>These findings highlight a previously unappreciated role for XMEF2C in the regulation of tendon development and identify a novel gene transactivation pathway where XMEF2C enhance the ability of the bHLH protein, scleraxis, to activate specific gene expression.</p>

PW34-430	<p><b><u>DROSOPHILA NEUROMUSCULAR JUNCTION (NMJ) PRUNING: A POTENTIAL MODEL TO FIND THERAPIES FOR NMJ DEGENERATION DISEASES</u></b>  BOULANGER A<sup>1</sup>, RAMANOUDJAME C<sup>1</sup>, DURA JM<sup>1</sup>  (1) Institut de Génétique Humaine CNRS, Montpellier, FRANCE.</p>
To contact the author:: aboulang@igh.cnrs.fr.	<p>At the neuromuscular junction (NMJ) individual muscle fibers are first contacted by many motoneurons, later in development synapse elimination and denervation followed by branch pruning occurs, so that, only one motoneuron innervates a same muscle fiber. In motoneuron diseases as ALS, one of the first signs of the diseases is also the denervation of neuromuscular junctions. Indeed, it is known that neurodevelopment and pathological neurodegeneration converge.  The aim of this study is to identify the mechanisms involved in motoneurons pathological degeneration using as a model the axonal dismantling of drosophila NMJ during development  In this study:  1) We have performed a precise description of the NMJ dismantling during drosophila development using immunohistochemistry, and shown that muscles degenerate before motoneurons. Thus, after muscle degeneration signs are observed, cell adhesion molecules are lost and the motoneuron cytoskeleton pulls-back by a retraction mechanism.  2) We have evidenced cellular (p35-mediated apoptosis) and genetic (ecdysone) pathways involved in NMJ dismantling. Accordingly, we have been able to stop NMJ dismantling by overexpressing or blocking specific ecdysone regulated nuclear receptors and by expressing apoptosis inhibitor proteins in muscle.  Together these results suggest that muscle degeneration is the trigger of the dismantling process since :  -muscle degeneration takes place before motoneuron pruning.  -Blocking muscle degeneration stops motoneuron pruning. However, blocking motoneuron pruning do not stop muscle lost.  We are actually trying to decipher the molecular nature of the message sent from the muscle to the motoneuron which induce motoneuron pruning. For this we are using a gene candidate screening approach.  Importantly, we have set up an in vivo developmental assay of NMJ dismantling in wich denervation can be stopped. The assay is ready to test the potential motoneuron or muscle degeneration-protective human genes, and can be used as a first test before gene therapy.</p>

PW34-431	<p><b><u>THE UCS FACTOR UNC-45B INTERACTS WITH THE HEAT SHOCK PROTEIN HSP90A DURING MYOFIBRILLOGENESIS AND SHUTTLES BETWEEN THE A-BAND AND THE Z-LINE OF THE MYOFIBRIL</u></b></p> <p>ETARD C<sup>1</sup>, ROOSTALU U<sup>1</sup>, BEHRA M<sup>2</sup>, STRÄHLE U<sup>1</sup>  (1) Institute for Toxicology and Genetics; Forschungszentrum Karlsruhe, Karlsruhe, GERMANY. (2) NIH/NHGRI, Bethesda, USA.</p>
To contact the author:: christelle.etard@itg.fzk.de.	<p>Contraction of muscles is mediated by highly organized arrays of myosin motor proteins. We report here the characterisation of a mutation of an UCS gene named <i>steif/unc45b</i> that is required for the formation of ordered myofibrils in both the skeletal and cardiac muscles of zebrafish. We show that Steif/Unc45b interacts with the chaperone Hsp90a <i>in vitro</i>. The two genes are co-expressed in the skeletal musculature and knock-down of Hsp90a leads to impaired myofibril formation in the same manner as lack of Steif/Unc45b activity. Our data indicate a requirement of Steif/unc45b and Hsp90a for the assembly of the contractile apparatus in the vertebrate skeletal musculature. In addition we show that Unc45b and Hsp90a co-localize with myosin during myofibrillogenesis and associate with the Z-line when myofibril assembly is completed. In response to stress or damage to the myofiber, Unc45b and Hsp90a dissociate from the Z-line and transiently associate with myosin. We propose that the Z-line serves as a reservoir for chaperones, allowing a rapid mobilization in response to muscle damage</p>

PW34-432	<p><b><u>COLLAGEN XXII, A NEW COMPONENT OF THE MYOTENDINOUS JUNCTION: A STRUCTURE-FUNCTION STUDY USING THE ZEBRAFISH MODEL.</u></b>  CHARVET B<sup>1</sup>, BADER H<sup>1</sup>, SCHULZE J<sup>2</sup>, KOCH M<sup>2</sup>, RUGGIERO F<sup>1</sup>  (1) Institut de biologie et chimie des protéines, UMR 5086, Université Lyon 1, IFR128 Lyon Biosciences, Lyon, FRANCE. (2) Institut for Biochemistry II, Medical Faculty of the university of Cologne, Cologne, GERMANY.</p>
To contact the author:: b.charvet@ibcp.fr.	<p>The myotendinous junction (MTJ) provides a structural link between the muscle cell cytoskeleton and the extracellular matrix (ECM) of tendons. Lack of one of the MTJ components can alter dramatically muscle cell anchoring to the tendon. Still, its morphogenesis remains elusive and has been neglected by biologists.</p> <p>Collagen XXII is a novel component of MTJ (Koch <i>et al</i>, 2004). It belongs to the FACIT (Fibril-associated collagen with interrupted triple helices) subset of the collagen family which are characterized by their capacity to associate with the fibrillar collagens and to mediate protein interactions. We aim at analyzing the role of collagen XXII in MTJ morphogenesis in zebrafish. Visual direct observations of embryos and rapid and efficient protein knock-down unmatched in other animal models make zebrafish a particularly valuable model for exploring muscle/tendon development. We identify the zebrafish ortholog of the human collagen XXII gene (<i>COL22A1</i>) and show that its transcripts are exclusively expressed in myotomal muscle at 24 hpf. As muscle cells differentiate, the signal concentrates at the extremities of muscle fibers close to the MTJ and sandwiches the myosepta, the structure equivalent to mammalian tendons. Confocal double immunofluorescence with antibodies to dystrophin and to zebrafish collagen XXII shows that collagen XXII is deposited at the MTJ. Morpholino-based knock-down of <i>col22a1</i> causes bending of the tail and important morphofunctional alterations in muscles/tendon formation: myosepta interruptions and presence of giant muscle cells spanning two myotomes at the interruption sites, muscle fiber detachment from the MTJ... Several mutations in ECM genes have been associated to myopathies. The genetic heterogeneity of the disorders suggests that other matrix proteins could account for unexplained cases. Our results suggest that collagen XXII plays a crucial role in muscle homeostasis and thereby represents a good candidate gene for muscular dystrophies.</p>

PW34-433	<p><b><u>APPROACHING THE MECHANISMS UNDERLYING THE EARLY LOSS OF LIMB MUSCLE FUNCTION IN ACUTE QUADRIPLEGIC MYOPATHY USING A PORCINE ANIMAL MODEL</u></b>  OCHALA J<sup>1</sup>, BANDUSEELA V<sup>1</sup>, LARSSON L<sup>1</sup>  (1) Uppsala University, Uppsala, SWEDEN.</p>
To contact the author:: julien.ochala@neurofys.uu.se.	<p>Acute quadriplegic myopathy (AQM) is commonly observed in patients suffering from critical illness, and is notably characterized by an early severe limb muscle weakness. The basic mechanisms underlying such muscle weakness remain poorly understood. It may be related to various components, i.e., sepsis, prolonged mechanical ventilation, postsynaptic block of neuromuscular transmission (NMB), systemic corticosteroid hormone treatment (CS), and muscle unloading. The present study aimed at exploring the relative importance of each component on the early development of limb muscle weakness using a porcine model. 21 pigs were mechanically ventilated and exposed to various combinations of NMB, CS and/or endotoxin-induced sepsis, for a period of five days. Biopsy specimens from biceps femoris on day 1 and 5, allowed the measurements of muscle function (single muscle fiber preparation), protein (12% SDS-PAGE gels, ELISA assay) and mRNA (Real-time PCR) expressions. Results showed interesting findings pointing out the major components that seriously accounts for the early development of AQM. On day 5, single muscle cell force-generating capacity was dramatically altered, the severity was related to the exposition to the various components: combination of sepsis, CS and NMB &gt; sepsis &gt; CS &gt; NMB. This impairment may be directly related to the lower expression of contractile proteins, related to degradation in the early phase of the disease.</p>