

**PW 4:
DMD physiopathology**

PW4-043	<p><u>RESTING CA²⁺ INFLUX AND POTENTIAL ROLE OF TRPC CHANNELS INVESTIGATED BY MN²⁺ QUENCHING OF FURA-2 FLUORESCENCE AND GENE TRANSFER IN MAMMALIAN SKELETAL MUSCLE FIBRES UNDER VOLTAGE CONTROL</u> BERBEY C¹, WEISS N¹, LEGRAND C¹, ALLARD B¹ (1) Université Lyon 1, UMR CNRS 5123, Villeurbanne, FRANCE.</p>
To contact the author:: bruno.allard@univ-lyon1.fr.	<p>A Ca²⁺ influx is known to occur in skeletal muscle cells at rest. The study of this influx is of primordial interest since an exacerbated resting Ca²⁺ entry has been considered to represent an early step associated with the pathophysiological changes in dystrophin-deficient muscles. Single channel recordings have revealed the existence of Ca²⁺ channels open at rest that could correspond to TRPC channels since repression of expression of these channels led to a reduction of channel activity. At the macroscopic level, the passive entry of Ca²⁺ has been essentially monitored using the Mn²⁺ quenching method but this was always performed in the absence of voltage control of the cell and therefore it was not established whether Mn²⁺ entry generates a measurable macroscopic membrane current consistent with the existence of Ca²⁺ permeable ion channels open at rest. The aim of the present study was to investigate resting Ca²⁺ influx using the method of Mn²⁺ quenching of fura-2 fluorescence on enzymatically isolated mouse muscle cells under voltage control and the potential role of TRPC1 in this influx using an overexpression strategy. The rate of quenching of fura-2 fluorescence induced by substitution of Mn²⁺ for external Mg²⁺ was found to be dependent on the external [Mn²⁺] and on the cell membrane potential. Replacement of Mn²⁺ by Mg²⁺ gave rise to an outward current at -80 mV associated with an increase in the input resistance. Calibration of the fura-2 response in ionomycine-permeabilized cells indicated that the Mn²⁺ influx is too minute to be resolved as a macroscopic current. In cells overexpressing TRPC1 channels after gene transfer by electroporation, resting potential, action potentials, resting conductance and Mn²⁺ quenching rate of fura-2 were found to be unchanged. The possibility that TRPC1 channels are involved in other steps of muscle Ca²⁺ handling is currently explored.</p>

PW4-044	<p><u>EFFECT OF CALPAIN/PROTEASOME INHIBITION ON CALCIUM DEPENDENT PROTEOLYSIS AND MUSCLE HISTOPATHOLOGY IN THE MDX MOUSE</u> BRIGUET A¹, ERB M¹, COURDIER-FRUH I¹, BARZAGHI P¹, SANTOS G¹, HERZNER H¹, LESCOP C¹, SIENDT H¹, HENNEBOEHLE M¹, WEYERMANN P¹, MAGYAR J¹, DUBACH-POWELL J¹, METZ G¹, MEIER T¹ (1) Santhera Pharmaceuticals, Liestal, SWITZERLAND.</p>
<p>To contact the author:: guenther.metz@santhera.com.</p>	<p>Dystrophin deficiency is the underlying molecular cause of progressive muscle weakness observed in Duchenne muscular dystrophy (DMD). Loss of functional dystrophin leads to elevated levels of intracellular Ca²⁺, a key step in the cellular pathology of DMD. Calpains are activated in dystrophin-deficient muscle, and their inhibition is regarded as a potential therapeutic approach. The contribution of the ubiquitin-proteasome system in muscle wasting conditions and the finding that its pharmacological inhibition mitigates the dystrophic phenotype in mdx mice qualify the proteasome as a potential target for therapeutic intervention in DMD.</p> <p>Here we present evidence that uncontrolled Ca²⁺ influx into muscle cells similar to that observed in dystrophin-deficient muscle not only initiates calpain- but also proteasome-mediated proteolysis. Based on this finding we set out to optimize novel dual small-molecule inhibitors that inhibit both calpain as well as the proteasome in a cellular system with impaired Ca²⁺ homeostasis. Such dual calpain/proteasome inhibitors, when administered to mdx mice, significantly improve quantitative histological parameters.</p> <p>For further assessment of the role of calpain inhibition, we crossed mdx mice with transgenic mice overexpressing the endogenous calpain inhibitor calpastatin. While our data show that proteolysis by calpain is strongly inhibited in the transgenic mdx mouse, this calpain inhibition alone does not ameliorate muscle histology.</p> <p>In conclusion, these results indicate that inhibition of the proteasome rather than calpain inhibition alone is required to show histological improvement in dystrophin deficient muscles.</p>

PW4-045	<p>MUSCLE ATROPHY AND REDUCED FUNCTION IS ASSOCIATED WITH A DECREASED REGENERATIVE CAPACITY IN VENERABLE (OLD) DYSTROPHIC MDX MICE</p> <p>MOUISEL E¹, HOURDÉ C¹, VIGNAUD A¹, BUTLER-BROWNE G¹, FERRY A¹ (1) Inserm U787, UPMC Institut de Myologie, paris, FRANCE.</p>
<p>To contact the author:: ferry@chups.jussieu.fr.</p>	<p>Aging results in a deterioration of muscle structure and function in dystrophic mdx mice lacking dystrophin. In the present study we wanted to know whether this aggravation is associated with an age related decline in regenerative capacity. Both uninjured and experimentally injured muscles (Tibialis anterior) of mdx mice aged 4 weeks, 5, 12 and 18 and 24 months were studied. Right limb muscles of mdx mice were injured by cardiotoxin and compared to the untreated contralateral muscles. Our results show that muscle weights and maximal tetanic force of the uninjured muscles of the 18-24 month old mdx mice were lower when compared to 5 month old mdx mice. Moreover, following cardiotoxin injury the degree of recovery of maximal tetanic force and muscle fibre cross-sectional area were reduced in the 18-24 month old mice compared to the 5 month old mice. The possibility that these changes were associated with alterations in Akt/mTOR signalling pathway was also studied. In conclusion, our results indicate that the age-induced loss of muscle function and structure in mdx mice muscles is associated with a decline in the regenerative potential after experimental injury.</p>

PW4-046	<p>DECREASED PROTEIN PHOSPHORYLATION IN THE PI3K/AKT AND ERK SIGNALING PATHWAYS IN SKELETAL MUSCLES OF DYSTROPHIC DOGS FERON M¹, ROUGER K², DUBREIL L², ARNAUD MC¹, MEGENEY L³, SAKANYAN V⁴, GUEVEL L⁵</p> <p>(1) CNRS UMR 6204, Laboratoire de Biotechnologie, Faculté des Sciences et des Techniques, Nantes, FRANCE. (2) INRA UMR 703, Ecole Nationale Vétérinaire, Nantes, FRANCE. (3) Ottawa Health Research Institute, Ottawa, CANADA. (4) Protneomix, 2 rue de la Houssinière, Nantes, FRANCE. (5) CNRS UMR 6204 and OHRI, Nantes/Ottawa, FRANCE.</p>
To contact the author:: marie.feron@etu.univ-nantes.fr.	<p>Duchenne muscular dystrophy (DMD) is the most common and severe form of muscular dystrophy. The pathology is caused by mutations in the dystrophin gene but the mechanisms linking the absence of dystrophin to the massive muscle necrosis and progressive muscle weakness observed are not yet fully understood. The data accumulated suggest that signaling pathway deregulation may well play a role and we decided to focus on signaling pathways in skeletal muscles, using the Golden Retriever Muscular Dystrophy (GRMD) dog that has a pathophysiology reminiscent of that of human DMD. Antibody array analysis revealed a reduction in the phosphorylation of kinases in the PI3K/Akt and ERK signaling pathways in dystrophic when compared to healthy muscles. Analysis of the compartment-specific location of Akt indicated that this kinase, despite displaying moderately increased expression, exhibited less susceptibility to being bound to the plasma membrane and then being translocated into the nucleus. We found that low Akt phosphorylation was associated with increased expression of PTEN and PP2A phosphatases. The reduced amount of phosphorylated Akt affects downstream located pathways, as we observed decreased phosphorylation of both p70S6K and GSK3β kinases. Moreover, the amount of the non-phosphorylated and active form of GSK3β was elevated in dystrophic muscles. Decreased phosphorylation of ERK1 and ERK2 kinases was also demonstrated. Altogether, these findings suggest that the abnormalities identified in signaling pathways might lead to an imbalance between protein synthesis and protein degradation, thereby contributing to the complex physiopathological process observed in the GRMD dog.</p>

PW4-047	<p><u>MUSCLE-SPECIFIC MICRORNAS (MIRNAS) IN DUCHENNE MUSCULAR DYSTROPHY (DMD)</u> MACIOTTA S¹, MEREGALLI M¹, FARINI A¹, BELICCHI M¹, PAROLINI D¹, BRESOLIN N¹, TORRENTE Y¹ (1) Fondazione IRCCS Policlinico of Milan, Department of Neurological Science, University of Milan, Dino Ferrari Center, Milan, ITALY.</p>
To contact the author:: stemlab@ibero.it.	<p>DMD is caused by frameshift mutations in the gene encoding for dystrophin. These mutations are responsible for the loss of function of the dystrophin protein that leads to membrane destabilization and subsequent activation of pathophysiological processes. Clinically this situation leads to rapidly progressive and severe skeletal muscle weakness. Recently it has been discovered that a restricted group of microRNAs (miR-1, miR-133, miR-181 and miR-206) are muscle-specific and seem to be involved in <i>in vitro</i>-induced myogenesis. Moreover a recent work based on microRNA Array Analyses reported a dysregulation in the expression levels of several miRNAs in 10 distinct forms of muscular dystrophies. These data suggest that some miRNAs may be involved in the pathogenesis of DMD phenotype. The aim of our study is to understand if there is a participation of these regulatory molecules in the pathogenesis of DMD. In order to verify this hypothesis, up to now we compared the expression levels of miR-1 and miR-133 by Northern Blotting and real-time-PCR between different muscle tissues of a dystrophic mouse model (MDX) and normal mouse (C57BL). We also evaluated by the same techniques the expression of these miRNAs in fetal and adult human muscle (MSH) from DMD and normal subjects. Our data evidence a higher expression of both miRNAs in MDX muscles and in MSH isolated from DMD patients. Further studies need to be done to better understand the biological function of the upregulation of these two miRNAs in pathological tissues.</p>

PW4-048	<p><u>DISTINCTIVE PATTERNS OF MIRNA EXPRESSION IN HUMAN MUSCULAR DISORDERS</u> EISENBERG I¹, ERAN A², LIDOV HG², KANG PB², KOHANE IS², KUNKEL LM¹ (1) Children's Hospital Boston - Harvard Medical School and Howard Hughes Medical Institute, Boston, USA. (2) Children's Hospital Boston - Harvard Medical School, Boston, USA.</p>
<p>To contact the author:: ieisenberg@enders.tch.harvard.edu.</p>	<p>The muscular disorders are a heterogeneous group of over thirty different inherited diseases characterized by muscle wasting and progressive weakness of variable distribution and severity, resulting in significant morbidity and disability. Although considerable progress has been made in our understanding of the overall complexity of the pathogenesis of the various muscular disorders, the underlying molecular pathways remain poorly understood. In light of their involvement in modulating cellular phenotypes we hypothesized that miRNAs might be involved in the regulation of the pathological pathways leading to muscle dysfunction. We describe a comprehensive miRNA expression profile aiming to identify new and modifying elements involved in the regulatory networks of muscle and the signature pattern of 185 miRNAs associated with ten common myopathological conditions in human. While five miRNAs (146b, 221, 155, 214, 222) were found to be consistently dysregulated in all samples analyzed in the study suggesting that these miRNAs are involved in a common underlying regulatory pathway among all diseases other miRNAs were identified to be dysregulated only in one given disease and not in any of the others thus pointing to their involvement in unique regulatory mechanism.</p> <p>The subsequent identification of potential target genes and the unraveling of biological signaling pathways involved in this regulatory level in these disorders, point to an additional dimension of regulation of muscle function mediated by miRNAs. Together with the tight post transcriptional regulation at the mRNA level identified in Duchenne and Miyoshi myopathy and specific mRNA:miRNA predicted interactions, some of which are directly involved in compensatory secondary response functions and others in muscle regeneration, these findings suggest an important role of miRNAs in the pathology of muscular dystrophy.</p>

PW4-049	<p><u>SUB-DOMAINS OF THE DYSTROPHIN ROD DOMAIN DISPLAY CONTRASTING LIPID-BINDING AND STABILITY PROPERTIES</u> LEGARDINIER S¹, HUBERT JF¹, LE BIHAN O¹, TASCAN C¹, ROCHER C¹, RAGUÉNÈS-NICOL C¹, BONDON A¹, HARDY S², LE RUMEUR E¹ (1) Université de Rennes 1, UMR6026, Rennes, FRANCE. (2) Université de Rennes 1, UMR6061, Rennes, FRANCE.</p>
To contact the author:: elisabeth.lerumeur@univ-rennes1.fr.	<p>Dystrophin is a muscle scaffolding protein that establishes a structural link between the cytoskeleton and the extracellular matrix. Despite the large body of knowledge about the dystrophin gene and its interactions, the functional importance of the large central rod domain remains highly controversial. It is composed of 24 spectrin-like repeats interrupted by four hinges that delineate three sub-domains. We express repeat 1 to 3 (R1-3) and repeat 20 to 24 (R20-24) sub-domains, delineated by hinges 1-2 and 3-4 and the single repeats 2 (R2) and 23 (R23). We determine their lipid-binding properties, thermal and urea stabilities and refolding velocities. By using intrinsic tryptophan fluorescence spectroscopy and size exclusion chromatography, we show that R2 and the R1-3 sub-domain strongly interact with anionic phospholipids. By contrast, R23 and R20-24 sub-domain do not interact with lipids. Therefore, the region R1-3 makes up a lipid-binding domain (LBD1). In addition, the R1-3 sub-domain and R2 are dramatically less stable with T_m of 51 and 54°C compared to R20-24 sub-domain and R23 with T_m of 62 and 66°C. In addition, refolding velocities are profoundly different, with rate constants for R2 and sub-domain R1-3 being twice as high as for their counterparts R23 and sub-domain R20-24, respectively. The contrasting properties of the two sub-domains clearly indicate that they make up two specific structural units within the rod domain that are not interchangeable. Due to their lipid binding properties, spectrin repeats have been shown to be involved in the modulation of the mechanical stability of the red cell membrane. The observation of sarcolemma ruptures in dystrophin-deficient muscles provides evidence for dystrophin involvement in plasma membrane resistance during muscle contraction. According to their homology with spectrin, we propose that the lipid-binding properties of dystrophin sub-domain R1-3 are involved in the maintenance of sarcolemma stability.</p>

PW4-050	<p><u>ALTERATIONS IN TC10 ACTIVITY AND GLUCOSE METABOLISM ARE ASSOCIATED WITH DYSTROPHIC MUSCLE PATHOLOGY</u> EVANS C¹, LYNN M¹ (1) Ottawa Health Research Institute; University of Ottawa, Department of Cellular and Molecular Medicine, Ottawa, CANADA.</p>
To contact the author:: sistah_c@hotmail.com.	<p>Duchenne's muscular dystrophy (DMD) is a genetic muscle disorder that affects muscle fiber integrity. Cyclic phases of degeneration and regeneration eventually lead to necrosis and replacement of muscle with fibrotic tissue. The molecular events that attribute to the dystrophic phenotype in DMD have not been fully elucidated. One mechanism known to be involved is the kinase JNK. Kinase assays revealed elevated JNK activity in dystrophic (MDX) muscle compared to WT muscle. Similarly, the RhoGTPase TC10 may also be involved as we observed elevated TC10 activity in dystrophic (MDX) muscle compared to WT muscle. Previous studies have shown that both JNK and TC10 interact with specific components of the insulin signaling pathway and thus influence glucose metabolism. Interestingly, glucose metabolism has been reported to be altered in DMD patients. In the present study we investigated the expression pattern of proteins within the insulin signaling pathway in the murine (MDX) model of Duchenne's muscular dystrophy. Our observations revealed that in dystrophic (MDX) muscle, phosphorylation of the IRS-1 substrate at tyrosine⁹⁴¹, which is an essential requirement for normal insulin signaling, was decreased. In contrast phosphorylation of the IRS-1 substrate on serine³⁰⁷, which is affiliated with insulin resistance, was elevated. Moreover, JNK phosphorylates the IRS-1 substrate at serine³⁰⁷. Further downstream the insulin signaling pathway, translocation of GLUT4 to the plasma membrane facilitates glucose transport in response to insulin. Immunohistochemistry revealed that normal GLUT4 translocation was perturbed, as GLUT4 remained localized predominantly in the cytoplasm in dystrophic (MDX) muscle. However, GLUT4 remained primarily along the periphery of the plasma membrane in (WT) muscle. Additionally, PAS staining revealed an accumulation of glycogen levels in dystrophic muscle compared to WT muscle. These results suggest that perturbations in the insulin signaling pathway may contribute to the dystrophic phenotype.</p>

PW4-051	<p><u>EARLY ENDOMYSIAL FIBROSIS IS UNIQUELY ASSOCIATED WITH SEVERE FUTURE MOTOR OUTCOME IN DUCHENNE MUSCULAR DYSTROPHY (DMD)</u> DESGUERRE I¹, PORON F¹, BARBET P², GHERARDI R¹, CHRISTOV C¹ (1) INSERM U841-E10 (Institut Mondor de Recherche Biomédicale); Paris 12 University, Créteil, FRANCE. (2) Service d'Histologie, Hôpital Saint-Vincent de Paul, Paris, FRANCE.</p>
To contact the author:: romain.gherardi@hmn.a php.fr.	<p>In DMD, repeated cycles of acute myofiber necrosis and regeneration progressively lead to severe terminal myofiber degeneration and extensive fibrosis. A preliminary analysis of 39 muscle biopsies from DMD patients at different ages allowed rough time-course characterization of both interstitial and cellular alterations in DMD. It includes initial prominence of edema and fiber hypercontraction (2-4 years), followed by prominence of myonecrosis and regeneration (5-7 years), and then by fibrosis and fatty degeneration (8-10 years). However, there were marked inter-individual variations at both the myopathological and clinical level in patients of similar age. As DMD is more clinically heterogeneous than classically described and may involve fibrosis as a central pathogenic player (see the other abstracts by the same group), we carried out a multiparametric analysis of myopathological alterations and all available clinical variables in a series of 35 DMD steroid-free patients with both quadriceps muscle biopsy performed from 3 to 6 years and a long term follow-up (>10 years). Seven myopathological alterations (endomysial and perimysial fibrosis, myofiber size, myonecrosis, hypercontraction, endomysial oedema, and fatty degeneration) were assessed using Gomori trichrome and Collagen-1 immunostaining, stereological methods and color image segmentation applied to digitized images. Analysis was done with the Categories module of SPSS 11.0 software using non linear Categorical Principal Component Analysis, an approach allowing one to handle together nominal, ordinal, and interval variables, and suitable for data recorded with uncertain units (e.g. MMT scores). Plotting of myopathologic variables detected in the quadriceps muscle with motor scores obtained in the same muscle group (mean score, minimal score, score at 10 years, deterioration score), allowed us to point out endomysial fibrosis as the sole myopathologic alteration correlated with motor scores. We conclude that early muscle fibrosis represents a sign of poor future motor outcome.</p>

PW4-052	<p><u>ENDOMYSIAL FIBROSIS IN DUCHENNE MUSCULAR DYSTROPHY (DMD) IS ASSOCIATED WITH CAPILLARY ARTERIALIZATION, TORTUOSITY, AND INCREASED CAPILLARY-TO-FIBER DISTANCE</u></p> <p>DESGUERRE I¹, BARBET P², GHERARDI R¹, CHRISTOV C¹ (1) INSERM U841-E10 (Institut Mondor de Recherche Biomédicale); Paris 12 University, Créteil, FRANCE. (2) Service d'Histologie, Hôpital Saint-Vincent-de-Paul, Paris, FRANCE.</p>
To contact the author:: romain.gherardi@hmn.a php.fr.	<p>The primary muscle fiber injury in DMD is due to total absence of dystrophin. With evolution of the disease, an increasing extent of myofibrosis, the precise mechanisms of which remain to be fully determined, likely accelerates myofiber death. Ischaemia has long been considered a possible factor of myoinjury. It is currently attributed to functional vascular alterations resulting from smooth muscle cell dystrophin deficiency and from ectopic sarcoplasmic nNOS location impeding paracrine NO release that normally regulates vasoconstrictive responses to exercise. We examined here the possible relationships between fibrosis and myofiber ischaemia on an anatomical basis. Frozen transversal sections from 15 DMD patients (6–10 years) and normal age-matched controls, immunostained for endothelial (CD31) and smooth muscle (alpha-SMA) cells, were used to set-up large scale reconstructions containing hundreds of microvessels. Vessels, myofiber sections and endomysium were segmented out of the images, and the following parameters measured: 1) arteriolarization index (percentage of arteriolar to total vascular area), 2) number of capillaries/mm², 3) distance separating capillaries from their nearest neighbors, 4) size/size variation of capillary domains defined as part of the muscle section closer to one capillary than to any other, 5) capillary tortuosity (length of capillaries) and individual myofiber vascularization indices: (i) the individual capillary-to-fiber ratio which takes into account the sharing factor, (ii) capillary-to-fiber perimeter exchange index, (iii) capillary-to-fiber distance. On the one hand DMD muscle showed conspicuous arteriolarization and marked increase of the capillary-to-fiber distance with interspersed collagen deposits, forming two major obstacles to gas exchanges. On the other hand, there was marked increase of both capillary tortuosity and capillary-to-fiber perimeter exchange index, suggesting an adaptative neoangiogenic phenomenon associated with injury and/or hypoxia-induced myofiber regeneration (see Christov et al, Mol Biol Cell 2007). Apparently, microvascular hyperplasia is likely to precede capillary depletion seen in the terminal stages of the disease.</p>

PW4-053	<p><u>SHORT DYSTROPHIN PROTEIN COMPLEX CONTAINING GAMMA1 AND GAMMA2 SYNTROPHINS IN NUCLEI OF CEREBRAL CORTEX OF RAT.</u> CANDELARIO-MARTÍNEZ A¹, RODRIGUEZ MUÑOZ R¹, MORNET D², MARTÍNEZ-ROJAS D¹ (1) CINVESTAV, Physiology, Biophysic and Neurosciences Department., México D.F. México, MEXICO. (2) 2INSERM, Equipe ESPRI 25 « Muscle et Pathologies », Université de Montpellier I, UFR de Médecine, EA 701, Montpellier, France., FRANCE.</p>
To contact the author:: damartin@fisio.cinvestav.mx.	<p>Syntrophins are a family of scaffolding proteins with multiple domains that link signalling proteins to dystrophin family members (Dp71 in brain). Syntrophins can interact simultaneously with multiple proteins via two pleckstrin homology domains, a PDZ domain and a conserved syntrophin unique region. Some syntrophins (α, β1 and β2) have a PDZ domain that binds signalling proteins like kinases, ion and water channels, diacyl glycerol kinase and neural nitric oxide synthase (nNOS). Previously, a short dystrophin associated complex (Dp71~DAPC) was characterized in nuclei from hippocampal neurons (Alemán et al, in press). On the other hand gamma1 syntrophin was found highly expressed in nuclei from neurons (Pilusso et al, 2000).</p> <p>In this work, we characterized the Dp71~DAPC containing γ1 and γ2 syntrophins and their intranuclear distribution in nuclei from cerebral cortex of rat.</p> <p>We found α, β, γ1 and γ2 syntrophins are expressed in nuclei and they are enriched in nuclear matrix fracction. By immunoprecipitation a similar pattern of proteins associated to γ1 and γ2 syntrophins was found. The distribution of α and β syntrophins determined by confocal microscopy was speckles-like structures, while γ1 and γ2 syntrophins localization was granular. In conclusion the Dp71~DAPC containing γ1 and γ2 syntrophins are localized in nuclear matrix and bound to some signal proteins.</p>

PW4-054	<p>DYSTROPHIN DP71 LOCALIZATION IN THE RETINAL VASCULAR SYSTEM BENARD R¹, DUPAS B¹, TADAYONI R¹, SENE A¹, ROUX MJ¹, NUDEL U², YAFFE D², SAHEL JA¹, RENDON A¹ (1) Université Pierre et Marie Curie-Paris6, INSERM UMR-S 592, Institut de la Vision, Paris, FRANCE. (2) Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, ISRAEL.</p>
To contact the author:: romain.benard@st-antoine.inserm.fr.	<p>Dp71 is the most abundant Duchenne Muscular Dystrophy (DMD) gene product expressed in the retina. This protein in the Müller glial cells (MGC) plays a role in regulating the retinal homeostasis by clustering Kir4.1 and AQP4 channels. (see poster of Sene A.) Our purpose here was to perform the study of the Dp71 expression in the retinal vascular system.</p> <p>The experiments were performed in mouse adult retinas of wild type (wt) and Dp71-null mice strains, on transverse sections and retina flatmounts. Direct evaluation of endogenous Dp71 promoter activity was performed by X-Gal staining. Dp71 and specific cell markers of vascular system expression was evaluated after double labelling with a dystrophins antibody (H4), and the following antibodies specific for astrocytes (GFAP), endothelial cells (isolectin B4), MGC (glutamine synthetase) and microglia (Cd11b).</p> <p>On retinal flatmounts of Dp71-null mice, X-Gal staining follows the vascular pattern, suggesting that Dp71 was localized inside and/or around retinal blood vessels. Closer magnification revealed a stain in cells resembling pericytes. On wt retinal flatmounts, H4 staining was localized around blood vessel wall and also in pericytes. This labeling disappeared in Dp71-null mice, suggesting that Dp71 was the only DMD gene product expressed. No H4 labeling was found in microglia or in endothelial cells of wt. On wt retinal sections, immunostaining with the H4, glutamine synthetase and the GFAP antibodies showed that Dp71 was present in the endfeet of MGC and at the internal limiting membrane, but also in astrocytic endfeet surrounding blood vessels. Surprisingly, amacrine neurons were also positive for H4.</p> <p>These results revealed novel cellular expression of Dp71 in the retina. Dp71 is not only present in MGC, but also in astrocytes (as in the brain), pericytes and amacrine neurons. These locations invest Dp71 with a potential broad spectrum of implications in the physiopathology of the retina.</p>

PW4-055

**FUNCTIONAL DIFFERENCES OF THE DP71- AND UTROPHIN-DAPS
COMPLEXES IN RETINAL MÜLLER CELLS : ROLE OF MEMBRANE
MICRODOMAINS.**

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We have showed that Dp71 and Utrophin are the only dystrophin superfamily members expressed in Müller Glial cells (MGC). Dp71 deletion leads to a compensating utrophin up-regulation.

Here we characterized the Dp71 or Utrophin /Dystrophin Associated Protein (DAPs) complex responsible for the localization and clustering of the ionic and water channels Kir4.1 and AQP4 in MGC membrane microdomains.

Dp71, Utrophin, DAPs, Kir4.1 and AQP4 were localized by immunocytochemistry in dissociated MGC from wild-type (wt) and mice invalidated for Dp71. The components of DAPs complex were identified by coimmunoprecipitation using membranous fractions enriched in Dp71; the end feet of MGC. Furthermore their membrane association was studied by analyzing their Triton X-100 solubility.

Observation of freshly dissociated MGC showed that Dp71, Utrophin, DAPs, Kir4.1 and AQP4 were localized on the end feet of MGC from wt mice. In Dp71-null mice, Utrophin, DAPs, Kir4.1 and AQP4 were delocalized all along MGC. We also found that Dp71 and Utrophin were associated to DAPs (β -dystroglycan, α -dystrobrevin and syntrophins), Kir4.1 and AQP4. We showed that Dp71 and DAPs were recovered in a detergent resistant membrane (DRM) fraction and in a soluble fraction in wt mice. Concerning Kir4.1 and AQP4, these proteins were recovered respectively in the soluble and the DRM fraction. Utrophin was recovered in soluble fraction in wt and Dp71 null mice. In Dp71 null mice, we observed that all DAPs were fully recovered in the soluble fraction; Kir4.1 remains in the soluble fraction and the expression of AQP4 was down regulated.

These results demonstrate that Dp71 has a central role in the molecular scaffold responsible for anchoring AQP4 and Kir4.1 in MGC end-feet membranes. Our results also suggest that despite its close relationship to the dystrophin proteins and its correlated up-regulation, utrophin is only partially compensating for the absence of Dp71 in MGC.