12èmes Journées de la Société Française de Myologie et Colloque Myogenèse

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AVANCÉES RÉCENTES DANS LE DOMAINE DES MALADIES MÉTABOLIQUES

PARIS - GHU PITIÉ-SALPÊTRIÈRE - 20/21 NOVEMBRE

2014 RECUEIL DE COMMUNICATIONS

SESSIONS DE COMMUNICATIONS

ORALES COLLOQUE MYOGENESE

Session 1 - Jeudi 20 Novembre 14h/16h30

N° 1#GB17 - Role of CXCL12/SDF-1 during muscle regeneration

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CXCL12 bind the receptor, CXCR4, the interaction of CXCL12 with the glycanic moiety of proteoglycans, in particular with heparan sulfates (HS), is believed to contribute to the biological activity of the chemokine by enabling the formation of CXCL12 gradients that determine the oriented migration and tissue recruitment of circulating cells. Nothing is known about the role of CXCL12 during muscle regeneration. The role of CXCL12 during muscle regeneration via the muscle stem cells (satellite cells) has never been described. The purpose of this study is to identify the role of CXCL12 in satellite cells niche in normal and pathological condition using a transgenic mouse line recently developed where the gene encoding CXCL12 was selectively mutated at its binding site HS thus preventing CXCL12-HS bond. The first results show that in the mutant mice, the morphology of the adult muscle is normal. In contrast, the muscle regeneration is severely affected with severe muscle fibrosis and lipid accumulation. The same phenotype is observed in human myopathies. The satellite cell compartment doesn't show structure or behavior abnormalities, however, the endothelial cell compartment has a major vascularization defect with many anastomotic collateral and a large number of cells forming filopodia called "tip cells".

Mot(s) Clé : muscle regeneration, Stem cells

N° 2#GB4 - Pericytes in the myovascular niche promote post-natal myofiber growth and satellite cell quiescence

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The satellite cells, which serve as adult muscle stem cells, are both located beneath myofiber basement membranes and closely associated to capillary endothelial cells. We observed that 90% capillaries are associated with pericytes in adult mouse and human muscle. During post-natal growth, newly formed vessels with their NG2+ pericytes became progressively associated with the post-natal muscle stem cells as myofibers increased in size and satellite cells entered into quiescence. In vitro, human muscle-derived pericytes promoted myogenic cell differentiation through IGF-1, and myogenic cell quiescence through Angiopoietin-1. Diphtheria toxin-induced ablation of muscle pericytes in mice led to myofiber hypotrophy during post-natal growth, associated with impaired establishment of stem cells quiescence. Similar effects were observed following conditional in vivo deletion of IGF-1 or Angiopoietin-1 genes in pericytes. Our data therefore demonstrate that, by promoting post-natal myogenesis and stem cell quiescence, pericytes play a key role in the microvascular niche of satellite cells.

Mot(s) Clé : Post-natal development, Satellite cell differentiation

N° 3#GB1 - Elderly muscle stem cells forget to go back to sleep

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Ageing affects stem cell number and function through a combination of extrinsic and intrinsic actors, but the mechanisms involved are not well understood. Here, taking muscle stem cells as a model, we investigated by which mechanism this population is progressively diminished with age. In cells from elderly subjects, we observed an impaired capacity for self-renewal combined with a shift towards differentiation into new muscle tissue and away from the replenishment of the reserve cell pool. Commitment towards a reserve cell fate was regulated by differential DNA methylation of genes that drive quiescence, such as sprouty1 (SPRY1). The capacity of cells to replenish the reserve cell pool could be increased or decreased experimentally by demethylation or siRNA knockdown of SPRY1. We propose that down-regulation of SPRY1 by DNA hypermethylation in ageing humans inhibits the replenishment of the muscle stem cell pool, contributing to a decreased muscle regenerative response in old age.

Mot(s) Clé : Sprouty1, Human muscle stem cell

N° 4#GB16 - RIBONUCLEASE T2 (RNAseT2) AS A NEW MOLECULAR ACTOR OF SKELETAL MUSCLE FUSION

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Macrophages are crucial cell players known to adapt their phenotype and secretory profiles (pro inflammatory (M1) vs anti inflammatory (M2) status) to sustain skeletal muscle regeneration. M1 macrophages promote myogenic precursor cell proliferation and inhibit early differentiation whereas M2 stimulate the commitment into the terminal differentiation myogenic program and the fusion of differentiated myogenic cells.
Using bioinformatic tool, we identified RNAseT2 an extracellular secreted glycoprotein preferentially expressed by M2 as compared with M1 macrophages.
With the use of a co-culture setup (conditioned media of macrophages on myoblasts) and loss of function silencing experiments, we showed a lower fusion index of myoblasts treated with conditioned media of RNAseT2-silenced macrophages, suggesting that this protein is specifically involved in the fusion step of myogenic cells.
Finally, we performed in vitro gain of function experiments using two different recombinant proteins: actibind, the native form RNAseT2 protein and TRT250, a partially inactive protein lacking actin-binding properties. Treatment of myogenic cells showed that actibind increased the fusion index, whereas TRT250 treatment had no effect, suggesting that fusogenic properties of RNAseT2 are related to its actin-binding activity.
Here, we reported data illustrating the role of RNAseT2, a new molecular actor involved in skeletal muscle fusion.

Mot(s) Clé : Macrophages, Secreted, Protein

N° 5#GB7 - Self-renewal and metabolism of muscle stem cells are regulated by AMPKa1

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During skeletal muscle regeneration, a subset of muscle stem cells (MuSCs) will not enter into the

myogenesis but will self-renew to return into quiescence. The control of this process is crucial to maintain skeletal muscle homeostasis. Recent studies highlight the importance of the metabolism switch in the regulation of stem cell fate. In this way, the master metabolic regulator AMPK seems to be an excellent candidate in the control of stem cell fate choice.

Ex vivo, in vitro and in vivo experiments permit us to pointing out that in absence of AMPKa1 MuSCs differentiate in a lower extend and self-renew much more. Also, we highlight that skeletal muscle regeneration is impaired without AMPKa1 in MuSCs. A strong decrease in myofiber size is observed and a significant increase in the total number of fibers per muscle 28 days post injury is noticed. In vitro, our results support the hypothesis that in absence of AMPKa1 MuSCs are not able to use mitochondria to synthesize energy.

Our work permits to establish a new and crucial role of AMPKa1 in muscle stem cell fate choice by switching the metabolism during skeletal regeneration, linking for the first time self-renewal and metabolism in this context.

Mot(s) Clé : Self-renewal, AMPK

N° 6#GB24 - Muscle-resident stem cells in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a common human inherited disease characterized by degeneration of motoneurons (MN) and muscle atrophy. This pathology is due to mutations of the Survival of Motor Neuron 1 gene (SMN1) which codes for an ubiquitously expressed protein (SMN) involved in various cellular processes including cytoplasmic assembly of snRNP into the spliceosome and pre-RNA splicing, and more recently in stem cell function. We are involved in the development of gene therapy strategies for SMA based on the delivery of self-complementary AAV9-SMN1 vectors (scAAV9-SMN1). We and others have demonstrated that a single intravenous injection induced a tremendous rescue of SMNΔ7 mice, a model of SMA. However, despite the restoration of SMN expression in the central nervous system (CNS) and peripheral organs, this strategy did not allow the complete rescue of the treated mice (mean life expectancy ~200 days). Although SMA has traditionally been considered as a pure lower MN disease, growing evidences suggest that peripheral organs including skeletal muscle, are also vulnerable to reduced levels of SMN. In order to investigate a potential dysfunction of muscle-resident progenitors in SMA, we studied satellite cells and PW1+ interstitial progenitor cells (called PICs) in a severe mouse model of SMA (hSMN2). Our results show significant changes in the number of both populations in SMA mice muscles. In addition, we observed differentiation defects of these two populations of progenitor cells in vitro. Thus, our results strongly support a specific role for SMN in muscle-resident progenitor cells function.

Mot(s) Clé : Muscle Stem cells, SMN

N° 7#GB5 - Vascular network and its relation with satellite cells in normal muscle regeneration

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During muscle regeneration, myogenic precursor cells (MPCs) interact with neighbouring cells, including endothelial cells (ECs). Previous studies showed that specific interactions take place between vessel cells and MPCs. However, little is known about their impact on MPC fate and the underlying molecular mechanisms in normal and pathological muscle.

We demonstrated in vitro a functional interplay between ECs and MPCs as: i) both cell type attract each other in migration assay, suggesting the secretion of specific attractive factors; ii) ECs strongly stimulate MPC differentiation, iii) MPCs promote angiogenesis, i.e. differentiation of ECs vessel-like structures. These results were confirmed in vivo, in which MPCs specifically promote the formation of functional vessels in a dose-dependent way. These results show that myogenesis and angiogenesis take place together. Several molecular candidates regulating angiogenesis/myogenesis coupling, including transcriptomic analysis of ECs and MPCs sorted at different time points during muscle regeneration, are under investigation in functional assays.

Collectively our results show that specific interactions between MPCs and ECs couple myogenesis and angiogenesis during muscle regeneration. These interactions may be altered in degenerative myopathies, where we already demonstrated strong perturbations of the vascular network associated with functional alteration (weaker muscle perfusion).

Mot(s) Clé : Myogenic precursor cells, Endothelial cells

Session 2 - Jeudi 20 Novembre 17h45/19h30

N° 1#GB13 - HACD1, a regulator of membrane composition and fluidity, promotes myoblast fusion and is essential for skeletal muscle growth

Jordan BLONDELLE, Yusuke OHNO, Vincent GACHE, Alexandre PROLA, Stéphane GUYOT, Sébastien STORCK, Arnaud FERRY, Geneviève AUBIN-HOUZELSTEIN, Jean DEMARQUOY, Richard J. PIERCY, Stéphane BLOT, Akio KIHARA, Laurent TIRET, Fanny PILOT-STORCK
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The reduced diameter of skeletal myofibers is a hallmark of several congenital myopathies. However, the mechanisms underlying this defect remain elusive. In this study we investigate the role of HACD1, involved in elongation of long chain fatty acids, in muscle fiber formation. In humans and dogs, HACD1/PTPLA deficiency leads to a congenital myopathy with fiber-size disproportion and a generalized muscle weakness. Through analysis of HACD1-deficient dogs, mice, and C2C12 models, we provide evidence that HACD1 promotes myoblast fusion during muscle development and regeneration. We further demonstrate that differentiating myoblasts dynamically express a muscle-specific, Hacd1 full-length splice isoform encoding the only catalytically active protein, essential for myoblast fusion. Upon HACD1 induction, membranes of differentiating myoblasts were less rigid, contained increased concentrations of ≥C18 and monounsaturated fatty acids and decreased concentration of lysophosphatidylcholine, a potent inhibitor of myoblast fusion. Notably, adding of candidate fatty acids to HACD1-deficient myoblasts promoted their fusion. Our results suggest that muscle-specific splicing of Hacd1 increases myoblast membrane permissiveness to fusion via the dynamic modification of its lipid composition, thereby prompting muscle fiber growth. This work also highlights the possibility that defective myoblast fusion may play an important role in other congenital myopathies.

Mot(s) Clé : Congenital myopathies, Muscle development

N° 2#FR35 - Muscle niche ensures survival and reactivation of dormant Adult Muscle Precursor cells in Drosophila

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How stem cells specified during development keep their non-differentiated quiescent state, and how they are reactivated, remain poorly understood. Here we applied a Drosophila model to follow in vivo behavior of Adult Muscle Precursors (AMPs), which share several features with vertebrate muscle stem cells. We report that emerging AMPs display homing behavior, and that muscles act as their niche by protecting dormant AMPs from apoptosis. We observed that the AMPs contact muscle fibers by sending out thin

filopodia, a capacity that is essential for their spatial positioning. The key role of muscles in the AMP cell behavior is also observed at their exit from the quiescent state. We demonstrate that muscles send local inductive dllp6 signals, which at the end of second larval instar activate proliferation of AMPs. Unexpectedly, genetic rescue experiments reveal that the Insulin pathway acts upstream of Notch, and positively regulates proliferation of AMPs via dMyc. Thus we provide evidence for a niche-driven Insulin-Notch-dMyc cascade in setting the activated state of Drosophila AMPs, suggesting that it may also regulate the reactivation of vertebrate muscle stem cells.

Mot(s) Clé : muscle stem cell, Notch, Insulin pathway

N° 3#FR32 - Rev-erbα modulates skeletal muscle oxidative capacity

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The nuclear receptor Rev-erb-α modulates hepatic lipid and glucose metabolism, adipogenesis and the inflammatory response in macrophages. It is also a component of the biological clock and as such plays a role in the circadian control of metabolism. We asked whether Rev-erb-α controls skeletal muscle physiology. Using gain- and loss-of function studies, we show that Rev-erb-α is highly expressed in oxidative skeletal muscle and plays a role in mitochondrial biogenesis and oxidative function. Rev-erb-α-deficiency in skeletal muscle leads to reduced mitochondrial content and oxidative function, resulting in compromised exercise capacity. This phenotype was recapitulated in isolated fibers and in muscle cells upon Rev-erbα knock-down, while Rev-erb-α over-expression increased the number of mitochondria with improved respiratory capacity. Rev-erb-α-deficiency resulted in deactivation of the Lkb1–Ampk–Sirt1–Ppargc1-α signaling pathway, whereas autophagy was up-regulated, resulting in both impaired mitochondrial biogenesis and increased clearance. Muscle over-expression or pharmacological activation of Rev-erb-α increased respiration and exercise capacity. This study identifies Rev-erb-α as a pharmacological target which improves muscle oxidative function by modulating gene networks controlling mitochondrial number and function.

Mot(s) Clé : Rev-erb alpha, Skeletal muscle, oxidative function

N° 4#FR33 - Characterisation of signalling pathways controlled by glucocorticoids in skeletal muscles.

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Glucocorticoids (GC) are stress hormones that control glucose and lipid metabolism in various cell types, and exhibit potent anti-inflammatory properties. High levels of GC are known to induce degradation of muscle proteins into amino acids that serve as substrates for gluconeogenesis.

The activity of GC is mediated through the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily. Thus to unravel the signalling pathways controlled by GC in skeletal muscles, we generated mice in which GR is selectively ablated in skeletal muscle myofibers at adulthood. Our results demonstrate that GCs, via myofiber GR, decrease protein synthesis and promote protein degradation in skeletal muscles.

To identify the underlying molecular mechanisms, we performed on murine skeletal muscles transcriptomic analyses and chromatin immunoprecipitations followed by massive sequencing. Our data reveal that a number of genes involved in proteolysis, as well as in amino acid and lipid transport, were selectively up-regulated by GCs via myofiber GR, including some which were not previously reported to be glucocorticoid target genes in skeletal muscles.

Unravelling the cellular and molecular mechanism of GC-induced muscle atrophy will open new avenues to select GC with increased tissue selectivity, and identify new drug targets to combat muscle wasting.

Mot(s) Clé : Glucocorticoids, Atrophy, Mice model

N° 5#FR16 - BCMO1 as a potential target to improve skeletal muscle growth and repair

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The enzyme beta,beta-carotene-15,15' mono-oxygenase (BCMO1) cleaves provitamin A carotenoids into active vitamin A principally in liver and intestine. The BCMO1 gene is expressed at low level in the muscle tissue, including in myoblasts, but little is known about its function. In the chicken muscle, we observed that various bcmo1 expression levels are associated with different carotenoids contents. To investigate the potential role of BCMO1 on skeletal muscle, we assessed the impact of beta-carotene (BC), the prototype substrate of the BCMO1 enzyme, supplementation in vitro on proliferative primary avian myoblasts. Proliferation was evaluated by BrdU incorporation and by flow cytometry. The BrdU incorporation index was reduced and the proportion of G0/G1 cells increased following BC supplementation. Cell differentiation was evaluated by immunolabelling of sarcomeric myosin heavy chain (MHC). The proportion of sarcomeric MHC expressing cells and the differentiation index increased following BC supplementation despite the proliferative environment. The effects of BC were inhibited in the presence of DEAB, an inhibitor of retinaldehyde dehydrogenase. These results are in accordance with the hypothesis that the BCMO1 enzyme is active in myoblasts and can contribute to the retinoic acid production from BC. These data suggest that provitamin A could be used as a potential nutritional tool in dystrophic pathology to favor muscle repair or to improve the implantation of myoblasts in cell transplantation model.

Mot(s) Clé : BCMO1 enzyme, myogenesis

N° 6#GB12 - Impaired mitochondrial function and reduced energy cost as a result of severe muscle damage

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Neuromuscular electrostimulation (NMES) exercise induces severe muscle damage but the corresponding effects on muscle energetics remains to be determined. The aim of the study was to determine whether NMES exercise alters muscle energetics at rest and during a submaximal voluntary exercise. 31P magnetic resonance spectroscopy measurements were performed in thirteen healthy males during a standardized rest-exercise-recovery protocol before (D0), two days (D2) and four days (D4) after NMES exercise on knee extensors. Changes in kinetics of phosphorylated metabolite concentrations (i.e., phosphocreatine [PCr], inorganic phosphate [Pi] and adenosine triphosphate [ATP]) and pH were assessed to investigate aerobic and anaerobic rates of ATP production and energy cost of contraction (Ec). A significant decrease in resting pH was determined (-0.04 pH.unit and -0.03 pH.unit, at D2 and D4 respectively). [PCr] recovery rate decreased at D2 (-21%) and D4 (-23%) in conjunction with a diminished total rate of ATP production at D4 (-18%) mainly due to an altered aerobic ATP production. Paradoxically, Ec was decreased at D4 (-21%). Overall, severe muscle damage led to intramuscular acidosis in resting muscle and mitochondrial impairment in exercising muscle. Alterations of non-contractile processes and/or adaptive mechanisms might account for the decreased Ec.

Mot(s) Clé : Magnetic resonance spectroscopy (MRS), ATP production

Session 3 - Vendredi 21 novembre 10h30/12h15

N°1#FR20- CHARACTERIZATION OF A NEW MODEL FOR DUCHENNE MUSCULAR DYSTROPHY, A RAT Dmd/mdx

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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. Their small size also imposes limitations for some analyses. 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. Two lines of Dmd mutated-rats (Dmdmdx) were generated using TALENs. Animals of both lines showed undetectable levels of dystrophin by western-blot and less than 5 % of dystrophin positive fibers by immunohistochemistry in muscles analyzed. At 3 months, limb and diaphragm muscles displayed intense necrosis and regeneration. At 7 months, these muscles showed severe fibrosis and adipose tissue infiltration. At both time points, Dmdmdx rats showed significant reduction in muscle strength and a decrease in spontaneous motor activity. Furthermore, 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. In conclusion, Dmdmdx rats represent a promising small animal model that can now be used 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.

Mot(s) Clé : Duchenne Muscular Dystrophy, TALEN

N° 2#FR22 - REGULATION OF YAP AS SENSOR AND MEDIATOR OF MECHANICAL INPUTS FROM THE EXTRACELLULAR MATRIX IS SEVERELY IMPAIRED IN LMNA MUTANT MYOBLASTS

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A-type lamins (lamins A/C), encoded by the LMNA gene, are nuclear intermediate filaments that are key components in the molecular chain that connects the nuclear interior to the cytoskeleton. It has been proposed that A-type lamins play important role in the transmission of forces between the nucleus and the extracellular matrix (ECM). We recently reported severe mechanosensing defects in human myoblasts with various LMNA mutations (LMNA) causing severe muscular dystrophies. These defects are associated with an up-regulation of YAP, a transcriptional co-activator shown to be important regulator of the mechano-response. The present study aims to determine the mechanisms contributing to YAP deregulation in LMNA myoblasts. Treatment of myoblasts with latrunculin-A and blebbistatin that,

respectively, disrupt actin filament organization and inhibit myosin ATPase, induced a cytoplasmic relocalization of YAP in both control (WT) and LMNA myoblasts. cell-cell contacts, cell confinement, reduced cell spreading with nocodazole, or Y-27632 that inhibits Rho-associated protein kinase, induced the cytoplasmic relocalization of YAP in WT, but they did not affect the nuclear localization of YAP in LMNA. We analyzed the mRNA and protein expression of LATS1 and LATS2, the main upstream kinases responsible for YAP phosphorylation and cytoplasmic localization. Interestingly, the mRNA and protein expression of LATS2 were significantly higher in LMNA compared with WT whereas LATS1 expression did not differ between WT and LMNA. In conclusion, our results strongly suggested that the mechanical regulation of YAP as sensor and mediator of mechanical inputs from the ECM is severely impaired in LMNA mutant myoblasts.

Mot(s) Clé : mechanotransduction, lamins, YAP

N° 3#GB27 - MUSCULAR PHENOTYPE EXACERBATION OF THE DMSXL MICE, MODEL OF THE MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic neuromuscular disorder due to an unstable (CTG) repeat expansion in the 3'UTR of the DMPK gene. Mutated DMPK mRNA form nuclear foci and affect splicing regulation of various RNA transcripts. The DMSXL mouse model has been created with a large genomic fragment containing the human DMPK gene carrying >1000 CTG. This model mimics molecular, histological defects and most of DM1 phenotype. Nevertheless, as in DM1 patients, the mice's phenotype is variable and sometimes moderate. We aimed at worsening the muscular phenotype in DMSXL mice using forced eccentric exercise (downhill treadmill) to optimize the evaluation of future biotherapies efficacy. Our work suggests that the eccentric exercise can worsen the muscular weakness observed in DMSXL vs. WT, with a significant decrease of their specific maximal force (sP0) in gastrocnemius muscle. That is independently to body weight gain, muscle weight changes or HE staining histological abnormalities suggesting molecular deregulation pathways. Preliminary isoform quantification for candidate genes in WT gastrocnemius revealed that the splicing profile depend on state of development and could be affected for Ldb3 and Mbnl2 mRNA in non-exercised DMSXL vs. WT opening to further molecular investigations in exercised DMSXL.

Mot(s) Clé : Mouse model, Biotherapy

N° 4#GB25 - RECESSIVE SKELETAL MUSCLE SODIUM CHANNEL MUTATIONS UNDERLAY CONGENITAL MYASTHENIC SYNDROME-LIKE PHENOTYPE

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Mutations of the SCN4A gene encoding the pore-forming α-subunit of Nav1.4 classically cause dominantly-inherited myotonia and periodic paralysis. One severe case of congenital myasthenic syndrome (CMS) due to an apparently dominantly-inherited SCN4A mutation has been described but this relationship has never been confirmed. CMS is a clinically and genetically heterogeneous group of diseases with fatigable muscle weakness. They are due to impairment of neuromuscular transmission and result from mutations in genes encoding for proteins critical for the neuromuscular junction. We have identified a novel homozygous SCN4A mutation (p.R1454W) in a patient with a form of recessively-inherited CMS. Expression of p.R1454W mutant Nav1.4 in the human embryonic kidney 293 cells induced an important impairment of fast and slow inactivation compared to the wild type. In addition, the mutant channels have slower inactivation kinetics than the wild types. A slower current decay combined with a shift in channel availability at rest potentials can ultimately lead to membrane inexcitability and muscle weakness. Our data confirm that relationship between Nav1.4 and CMS-like phenotyped and question the clinical overlapping between periodic paralyses and CMS. We suggest that while Nav1.4 mutations exerting a dominant-negative effect cause periodic paralysis, a CMS-like phenotype may result from loss of function mutations that decrease the Nav1.4 availability for genesis of muscle action potential at the NMJ and its propagation along the sarcolemma.

Mot(s) Clé : congenital myasthenic syndrome, periodic paralysis

N° 5#GB26 - COLLAGEN VI DEFICIENCY LEADS TO ALTERED INTEGRIN ADHESION COMPLEX IN HUMAN SKELETAL MUSCLE

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Myopathies caused by the deficiency of collagen type VI (COLVI) constitute a large spectrum of clinical presentations ranging from severe, early-onset phenotypes (Ullrich congenital muscular dystrophy, UCMD), to intermediate and later-onset, milder forms (Bethlem myopathy). COLVI is expressed in most connective tissues, where it maintains structural integrity through multiple interactions with extracellular matrix (ECM), basement and cell membrane components. Impaired autophagy and increased apoptosis leading to altered myofiber survival and homeostasis have been implicated in the etiology of COLVI-myopathies, but the intermediary players between the COLVI-deficient ECM network and the intracellular compartment have remained elusive so far.

We analyzed the two main skeletal muscle adhesion protein complexes: the dystrophin-glycoprotein complex (DGC) and the integrin alpha7beta1, in muscle biopsies from a total of ten genetically confirmed UCMD patients and age-matched controls. By immunohistochemisty, immunoblotting and RT-qPRC, we ruled out involvement of the DGC, but demonstrated that the expression of alpha7beta1 integrin and several of its partners was significantly altered in COLVI-deficient muscle.

Our results provide further insight into the defective extracellular-intracellular signaling in COLVI-myopathies. The alteration of the integrin adhesion complex most likely plays an important role in triggering the downstream intracellular pathways that contribute to the muscle phenotype in these disorders, and may open new avenues for therapeutic options.

Mot(s) Clé : Extracellular Matrix, Integrin

N° 6#GB11 - MUTATIONS IN THE SOCE GENES STIM1, ORAI1 AND CASQ1 CAUSE TUBULAR AGGREGATE MYOPATHY (TAM)

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In skeletal muscle, Ca2+ triggers contraction and acts as a second messenger controlling growth and differentiation. Therefore, Ca2+ storage and release needs to be tightly regulated.

Tubular aggregate myopathy (TAM) is a progressive muscle disorder typically showing densely packed membrane tubules on muscle biopsies. We have recently identified STIM1 as the first TAM gene. STIM1 codes for the major Ca2+ sensor in the sarcoplasmic reticulum. Upon Ca2+ store depletion, STIM1 activates the Ca2+ entry channel ORAI1 to trigger extracellular Ca2+ entry. This mechanism of Ca2+ store refill is known as store-operated calcium entry (SOCE). Using exome sequencing, we have now found two further TAM genes, CASQ1 and ORAI1, both key factors in Ca2+ homeostasis. CASQ1 is a high-capacity calcium binding protein in the sarcoplasmic reticulum, and ORAI1 is a calcium channel in the plasma membrane. CASQ1 and ORAI1 mutations were found in three unrelated families each. Using in vitro and in cellulo approaches, we demonstrate that the mutations impact on the biological functions of the proteins and on intracellular Ca2+ balance. Importantly, all three TAM genes act within the same pathway regulating calcium balance, representing a strong proof for the implication of Ca2+ homeostasis alterations in the pathology.

Mot(s) Clé : calcium, STIM1

RAPPORT PRIX MASTER 2013

FONCTION ET MÉTABOLISME MUSCULAIRES CHEZ UN MODÈLE DE SOURIS DRÉPANOCYTAIRES : UNE EXPLORATION NON INVASIVE DU MUSCLE TRAVAILLANT

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La drépanocytose est une hémoglobinopathie génétique qui touche environ 50 millions de personnes dans le monde et constitue la première maladie génétique sur le globe. Elle se traduit par la synthèse d'une hémoglobine anormale (HbS) responsable de la falciformation et de la rigidification des globules rouges (GR). Ces GR falciformes ont tendance à obstruer la microcirculation induisant des crises vaso-occlusives (CVO) particulièrement douloureuses. La drépanocytose étant principalement considérée comme une maladie hémoglobinique et hémorhéologique, ses répercussions musculaires n'ont que très rarement été étudiées. Pourtant, la fatigue est un symptôme majeur de la maladie. De récents travaux montrent que la présence d'HbS est associée à un remodelage musculaire significatif témoignant d'un potentiel dysfonctionnement de l'approvisionnement et/ou de l'utilisation de l'oxygène par le tissu musculaire. Néanmoins, la traduction métabolique de ce remodelage au niveau du muscle travaillant est encore méconnue. L'objectif de ce travail est donc d'identifier les éventuelles anomalies fonctionnelles et métaboliques associées à la présence d'HbS au cours d'un protocole standardisé de repos-stimulation-récupération chez un modèle de souris drépanocytaires. Nous utiliserons deux protocoles à des fréquences de stimulation différentes afin de déterminer si les réponses à l'exercice sont différentes en fonction de son intensité.

RAPPORT PRIX MASTER 2012

COPING WITH CHOLINERGIC OVERSTIMULATION IS A MULTIMODAL PROCESS

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Myasthenia gravis is the most prevalent form of neuromuscular transmission troubles, and is often due to a downregulation of the acetylcholine receptors (AChRs) expressed at the muscle cell surface. Current treatments use acetylcholine esterase inhibitors to restore efficient neuromuscular transmission. Unfortunately these inhibitors can sometimes lead to a cholinergic overstimulation, called cholinergic crisis. To prevent these crises, alternative therapeutic strategies relying on the modulation of AChR metabolism or function are considered. So far, however, only few cellular factors are known to regulate AChRs.

As AChRs are conserved in many organisms, we use the model organism Caenorhabditis elegans to identify new mechanisms of AChR regulation. Adaptation to a chronic overstimulation of AChRs using a cholinergic agonist, levamisole, provides a suitable experimental approach: after an initial phase of paralysis, worms are able to recover locomotion and this phenomenon is correlated with a long-term downregulation of AChRs at synapses.

By monitoring the calcium homeostasis of muscle cells during adaptation, we show that the long-term adaptation is determined by early variations of calcium levels. In addition, we performed a large-scale genetic screen to decipher the signaling pathways involved in the regulation of AChRs. We raised a model for levamisole-mediated adaptation, in which the AChR cluster signaling is decoupled from the downstream mechanisms leading to global calcium variations. We suggest that a negative modulator being part of the clusters inhibits AChR function by shutting down the firing of action potentials.

Mots Clés: Acetylcholine receptors, neuromuscular junctions

POSTERS

N°1#FR13 - Séquençage haut débit de 142 gènes de myopathies en routine : apport pour le diagnostic clinique et moléculaire et pour la physiopathologie

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Environ la moitié des patients atteints de myopathies ne bénéficient pas d'un diagnostic moléculaire, en partie car tous les gènes impliqués ne sont pas encore identifiés, mais aussi en raison de l'hétérogénéité génétique et clinique et de la difficulté des analyses gènes par gènes pour tous les gènes connus. Nous proposons une approche de diagnostic en routine de 142 gènes de myopathies par capture de séquence et séquençage haut débit, testée dans le cadre d'un projet de recherche portant sur une cohorte de 150 patients enfants et adultes, adressés par les consultations du Centre de référence des maladies neuromusculaires du GrandEst. Les objectifs techniques incluent la détection de cibles complexes (expansions, gène dupliqué, anomalies de dosage) et un système de traçabilité des échantillons. Le projet se base sur un diagnostic clinico/moléculaire intégré avec les cliniciens et histopathologistes, en fonction des gènes/variants candidats, et sur un travail en réseau au sein des laboratoires de référence. Les résultats à mi projet illustrent la contribution de cette approche à un diagnostic moléculaire plus rapide et peu couteux, mais aussi aux connaissances sur les myopathies. L'élargissement des spectres cliniques et génétiques connus révèlent de nouvelles associations génético-phénotypiques et peuvent suggérer de nouveaux mécanismes pathologiques.

Mot(s) Clé : Séquençage haut débit, Diagnostic, Physiopathologie

N°2#FR32 - Analyse évolutive d'une cohorte de patients atteints de myopathie héréditaire à inclusions : de l'approche « gène par gène » à l'approche « Exome »

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Les myopathies héréditaires à inclusions (hIBM) représentent un groupe hétérogène de pathologies musculaires tant au niveau clinique que génétique. Ces hétérogénéités compliquent le diagnostic moléculaire actuellement réalisé avec les techniques de séquençage classique. Le Département de Génétique Médicale à Marseille effectue le diagnostic de routine d'une forme récessive d'hIBM, les myopathies héréditaires à inclusions associées au gène GNE (IBM2), en tant que laboratoire de référence national. Ainsi, au cours des 10 dernières années, 184 patients ont été inclus pour une analyse de GNE.

Parmi les patients analysés, un premier groupe constitué des 35 cas index diagnostiqués par séquençage classique pour une myopathie héréditaire IBM2 a permis de caractériser sur le plan moléculaire la première grande cohorte française de patients atteints d'IBM2, publiée à ce jour. De plus, nous avons pu décrire 13 nouveaux variants associés

aux myopathies impliquant le gène GNE, s'ajoutant aux 154 variants déjà référencés dans la dernière publication de Celeste et al. tout en évaluant l'effet potentiellement délétère sur l'épissage de l'ensemble de ces variants.
Le second groupe est constitué de 19 cas index sélectionnés parmi les patients non diagnostiqués après l'analyse par séquençage classique de GNE. Cette cohorte de patients atteints d'hIBM a été explorée par une approche de séquençage à haut débit (exome) nous permettant ainsi d'une part d'éprouver notre pipeline d'analyse et d'autre part de déterminer le diagnostic moléculaire chez 42% des patients. Ces travaux ouvrent la voie au diagnostic des hIBM par séquençage à haut débit.

N°3#GB28 - DM-SCOPE, a response to the complexity of myotonic dystrophies childhood clinical forms

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Myotonic dystrophy is one of the most variable neuromuscular disorder with its very large age of onset. This high variability creates particular challenges for management and the design of optimal therapeutic trial. The DM-Scope database, specifically dedicated to myotonic dystrophies, has been created to characterize a large population of patients, support research and propose guidelines in DM clinical Management. Since 2010, it includes applications to optimize the annual clinical evaluation of adult forms and for promoting clinical research. This year, we have specifically developed tools to improve the management of the paediatric forms and better characterized the Childhood DM1 disease (standardized form, synopsis…). Currently, twelve neuropaediatric centers participate to the observational study and overall the French registry gather data from 1923 adult and pediatric DM patients collected in 44 neuromuscular centers. This nationwide clinical network represents the largest DM cohort in the world. DM-Scope provides a powerful platform designed to optimize routine clinical management and clinical research in the field of myotonic dystrophies.

Mot(s) Clé : DM-Scope, Childhood clinical forms

N°4#GB31 - A large-sized genomic deletion in the DYSF locus causes Antley Bixler Syndrome due to deregulation of Retinoic Acid-dependent CYP26B1 expression.

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Antley Bixler Syndrome constitutes a very rare craniosynostosis syndrome characterized by radiohumeral synostosis genetically linked to mutations in the cytochrome P450 oxidoreductase gene (POR), the Fibroblast Growth Factor Receptor 2 gene (FGFR2), or CYP26B1 genes. Interestingly, clinical manifestations and mutated genes suggest retinoic acid (RA) signalization pathway involvement.
Here we report the case of a patient presenting with combined clinical manifestations of Antley Bixler Syndrome and myopathy of dysferlinopathy type. A homozygous deletion of ~160kb, localized between exons 2 and 40 of the DYSF gene on chromosome 2, has already been characterized in this patient. The CYP26B1 gene, which maps on minus strand of chromosome 2, is located 444kb downstream of the DYSF gene. Syntheny and relative gene positions are highly conserved through evolution. Strikingly, activation of CYP26B1 expression by RA is greatly reduced in lymphoblasts from the patient compared

to controls. Thus, the genomic DYSF deletion in the patient probably affects not only dysferlin protein function but also CYP26B1 gene expression explaining the clinical outcomes for this patient and implicating retinoic acid in the pathogenesis of Antley Bixler Syndrome.

N°5#FR4 - «Core-rod myopathy », entité d'une large hétérogénéité clinique et génétique

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La myopathie congénitale « core-rod » est caractérisée par la présence de ces deux anomalies morphologiques, les cores et les bâtonnets, retrouvées dans des zones distinctes de la fibre musculaire. Cette myopathie a été notamment associée à des mutations AD ou AR du gène RYR1, des mutations AD des gènes ACTA1 et KBTBD13, et à des mutations AR des gènes NEB et CFL2.

Nous présentons les caractéristiques cliniques, histopathologiques et génétiques d'une large série de familles/patients: a) 6 nouvelles familles avec des mutations dans le gène RYR1; b) 3 patients mutés dans le gène NEB; c) 6 patients pour lesquels aucune anomalie moléculaire n'a pu être identifiée dans les gènes connus. Ceci illustre le caractère hétérogène de cette entité.

Mot(s) Clé : myopathies congénitales, core-rod myopathy

N°6#FR7 - Functional analysis of ECEL1 missense mutations reveal at least two pathophysiological mechanisms implicated in ECEL1 related distal arthrogryposis.

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CONTEXT: Endothelin-converting enzyme like 1 (ECEL1) is a member of the neutral endopeptidase (neprilysine, NEP) family with a critical role in intramuscular axon branching of motor neurons during development. Recently we identified ECEL1 mutations in an autosomal recessive form of distal arthrogryposis. Most ECEL1 mutations are splice site or non-sense mutations predicted to produce truncated proteins or mRNA decay,therefore acting by a loss of function mechanism. Missense mutations are less frequent and their pathophysiological mechanism less well understood.

OBJECTIVE: Study the pathophysiological mechanism by which ECEL1 missense mutations lead to distal arthrogryposis.

METHOD: In vitro comparison of expression, subcellular localization and endopeptidase activity of wild-type and mutated ECEL1 proteins.

RESULTS: Our in vitro studies showed that all missense mutations we have identified so far modify neither expression nor the subcellular location of ECEL1. Our enzyme assay revealed loss of catalytic activity for one missense mutation that lies within the functional C terminal domain of ECEL1. Conversely, two other missense mutations that are near the N terminal domain did not affect the endopeptidase activity.

CONCLUSION: Just like non-sense and splice site mutations, we showed that loss of function is the pathophysiological mechanism for at least one ECEL1 missense mutation. However our results further suggest that other pathophysiological mechanisms

are involved in missense mutations lying outside the ECEL1 catalytic domain.

Mot(s) Clé : arthrogryposis, functional studies, ECEL1

N°7#FR30 - Traitemant d'un pied creux varus par injection de toxine botulinique chez une enfant atteinte de Maladie de Charcot Marie Tooth de forme récessive AR-CMT2.

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Contexte : La maladie de Charcot Marie Tooth (CMT) s'accompagne de déformations progressives en pied creux varus.
Observation : Une patiente de 11 ans porteuse d'une CMT de type 2 liée à une mutation du gène GDAP1 présentait un varus sévère du pied gauche . L'examen baropodométrique lors de la marche confirmait un hyperappui en regard de la base du cinquième métatarsien. Le varus était difficilement réductible.
Intervention: Une injection de 50 unités de toxine botulinique (Botox®) a été réalisée par voie échoguidée dans le muscle tibial postérieur gauche. Après quinze jours, l'arrière pied était plus souple, le varus du pied était légèrement amélioré lors de la marche et on notait une nette baisse de l'hyperappui plantaire externe (-35%). Sur le plan cinétique, l'affaiblissement du muscle tibial postérieur, entrainait une diminution de la force de propulsion à gauche (-36%) sans retentissement sur les paramètres spatio-temporels de marche.

Discussion : En limitant le déséquilibre musculaire entre le muscle tibial postérieur et les muscles valgisants déficitaires, le traitement par toxine botulinique a amélioré la statique du pied, rééquilibré les pressions plantaires, diminué les douleurs et limité la déformation en varus dynamique. Une étude à plus grande échelle serait nécessaire, notamment pour évaluer son rôle préventif sur la déformation orthopédique à long terme.

Mot(s) Clé : neuropathie de Charcot Marie Tooth

N°8#GB2 - mTOR is expressed in polymyositis but not in sporadic inclusion body myositis

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Objective. To refine the histological characterization of inflammatory exudates in polymyositis (PM) and sporadic inclusion body myositis (s-IBM).
Methods. We performed an immunohistochemical study with antibodies directed against the Sphingosine 1-Phosphate Receptor 1 (S1PR1), Formyl Peptide Receptor-type 2 (FPR2), mammalian target of rapamycin (mTOR) and classical markers of inflammation in 10 s-IBM, 10 PM and 8 control muscle biopsies.
Results. S1PR1 and FPR2 expression did not differ between the 3 groups, while mTOR was expressed in 7 out of 10 PM patients but in none of the s-IBM or control samples.
Conclusions. mTOR could potentially be used as a differential marker for PM inflammatory infiltrate and even represent a new potential drug target. Specificity and sensitivity of this marker need however to be further investigated.

Mot(s) Clé : Polymyositis, m_THOR pathways

N°9#GB21 - Ischemic inflammatory myopathy in a female patient with Fabry disease

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Patients with Fabry disease (FD, OMIM #301500) often report fatigue limb pain. Recent histopathological study indicates that skeletal muscle involvement occurs in FD, but milder and delayed compared with heart.
We report on a 28-year old heterozygous female with FD who first complained at 16 from pain in right facial muscles. Over 10 years, muscular pain spread to the neck, right upper limb and, lastly, lower limb with swelling of leg. Electromyogram was uninformative. Whole body muscle MR imaging disclosed STIR changes in anterior and posterior compartment of right leg suggestive of inflammatory injuries. Right tibialis anterior muscle biopsy was performed and showed non-necrotizing vasculitis associated with muscle infarct. In small arteries, endothelial cells and smooth muscle cells displayed focal PAS-positive and sudanophilic deposits suggestive of glycosphingolipid accumulation. Electron microscopy revealed typical lysosomal storage. Oral steroid therapy strikingly alleviated muscle pain but with notable cortico-dependence. Muscle MRI showed decrease of STIR hypersignals four months later.
This case showed that skeletal muscle involvement in FD can results from ischemic process in association with specific vasculopathy. Symptoms may be poorly specific and misleading and the combination of muscle MRI and histopathological investigation is necessary for the diagnosis.

Mot(s) Clé : inflammation, skeletal muscle,

N°10#FR14 - Evaluation de l'immunogenicité anti-rhGAA chez les patients adultes atteints de la maladie de Pompe

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La maladie de Pompe est une glycogénose musculaire lysosomale due au déficit de l'enzyme alpha-glucosidase acide (GAA). L'enzymothérapie substitutive (Myozyme®) a amélioré le pronostic de cette maladie, mais les réactions immunitaires contre l'enzyme recombinante (rhGAA) sont fréquentes et limitent l'efficacité thérapeutique. Le but de cette étude était d'examiner les mécanismes des réactions immunitaires contre le rhGAA chez des patients adultes traités. Nous avons démontré qu'environ un tiers de tous les sujets étudiés présentent des taux significatifs d'anticorps anti-rhGAA. Après maturation des cellules dendritiques en présence du rhGAA, une sécrétion d'IFN-gamma a été détectée en ELISpot, indiquant une réactivité spécifique des lymphocytes T. Nous avons également pu détecter une réponse chez des patients non traités. La re-stimulation in vitro a abouti à une sécrétion de cytokines proinflammatoires chez les sujets traités par Myozyme®, mais pas chez les sujets contrôles. Des études supplémentaires sont nécessaires pour mieux définir les sous-populations de cellules B et T impliquées dans ces réactions. Néanmoins, cette étude pilote fournit d'ores et déjà une vue complète de l'activation de l'immunité en réponse à l'enzymothérapie substitutive chez les patients Pompe. Elle offre des outils et des stratégies pour contrôler et gérer l'immunogenicité de cette biothérapie.

N°10bis#FR38 - Puissance diagnostique du test non-Ischémique de l'avant-bras : bilan après 15 ans d'expérience

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Le test non-ischémique de l'avant-bras (aussi connu sous le nom de Grip Test) a remplacé dans plusieurs consultations neuromusculaires le test ischémique (dit "du garrot") initialement proposé par McArdle en 1951 pour détecter les glycogénoses de type 5. La version ischémique a été effectivement jugée comme hasardeuse chez certains patients, notamment chez ceux atteints de la maladie de McArdle, car le risque de rhabdomyolyse n'est pas négligeable. En outre, du fait de la douleur causée par le travail musculaire sous ischémie, les patients interrompent leur effort de façon prématurée, ce qui génère un nombre significatif de faux positifs. Le Grip Test a été établi à l'Institut de Myologie en 1998. Il consiste à demander au patient de maintenir pendant 30 s un effort isométrique à 70% de la force de préhension maximale préalablement déterminée. Des échantillons sanguins prélevés de façon standardisée, généralement dans la veine basilique, permettent de suivre la lactatémie et l'ammoniémie au cours du temps.
Après près de 15 ans de pratique du Grip Test, les résultats de 1226 patients ont été rétrospectivement analysés. Chez plusieurs de ces patients, un diagnostic de glycogénose ou de mitochondriopathie a été posé. Plusieurs profils métaboliques ont été établis à partir des courbes de production de lactate et d'ammoniaque. Certains sont très typiques, comme cela peut être vu dans la maladie de McArdle; d'autres ont des décours plus subtils. Le thème de ces JSFM donne l'opportunité de dresser un bilan diagnostique du Grip Test.

N°11#GB22 - New mutation of PNPLA2 causes asymetric distal lipid storage myopathy and cardiomyopathy

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Neutral Lipid Storage Diseases (NLSD) is a group of diseases characterized by systemic accumulation of triglyceride. NLSD associated with myopathy has been reported: Chanarin-Dorfman syndrome, associated myopathie and ictyosis and mutation in PNPLA2 (Patatin domain containing phospholipase A2) responsible for systemic triglyceride accumulation in peripheral muscle, cardiac muscle, peripheral blood smear and liver.
We report the story of a 55 years old man who developed a dilated cardiomyopathy with episodes of ventricular tachycardia, slowly worsened, leading to a cardiac transplantation at 52 with a perfect result at 5 years.
At near the same time, he developed a serious asymmetric distal myopathy, predominantly in the left upper arm, without scapula alata, and in the left posterior compartment of the leg ; he had temporal muscle atrophy, no myotony.
EMG showed myogenic activities without myotony, CPK : 350.
Muscle biopsy in 2005 and 2012: optical and electronical microscopy showed lipidic storage myopathy . Mitochondrial biochemical activities were normal. Acyl carnitines analysis was normal.
Jordan bodies in blood were not observed. Cérébral MRI revealed no leucoencephalopathy.
A deleterious new mutation in PNPLA2 was finally detected: a homozygous mutation (c.798_799insC) in exon 7 of PNPLA2 (pAla 267Argfsx40), which was predicted to result in a premature stop codon.
The patient's two sisters report no medical history, but his younger brother recently developed heart arythmia.
According to a recent study, a treatment with bezafibrate 400mg/d was prescribed to the patient but was not tolerated, causing myalgia and CK elevation.

Mot(s) Clé : Cardiomyopathy, PNPLA2 Mutation,

N°12#FR9 - Mutations RYR1 et Rhabdomyolyse aigue chez l'adulte

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Méthode : Nous avons étudié 14 adultes ayant présenté une rhabdomyolyse aigüe (CK>10000 UI/l), chez lesquels la maladie de Mac Ardle, une anomalie de l'oxydation des acides gras et une mutation de LPIN1 avaient été exclues. Tous les patients ont eu une biopsie musculaire.

Résultats : une mutation potentiellement pathogène du gène RYR1 a été détectée chez 5 patients sans lien de parenté. Les mutations étaient : p.Val4847Leu, p.G593R, p.R3539H, Gly2434Arg, p.Gln3461Pro, p.A1352T, p.A933T et p.Ser1342Gly. Les CK de base étaient élevées chez tous les patients sauf un. Aucun d'entre eux ne se plaignait de myalgies ou d'intolérance à l'exercice. Les épisodes de rhabdomyolyse étaient déclenchés par l'effort, une virose et un traitement par acide rétinoïque. Le taux maximal de CK était entre 28000 et 94000UI/l. Les biopsies musculaires étaient soit normales, soit en faveur d'une désorganisation de structure modérée. Un épisode d'hyperthermie maligne peranesthésique a été rapporté chez la grand-mère d'un des patients.

Conclusion : les mutations du gène RYR1 devraient être recherchées chez les patient présentant un épisode de rhabdomyolyse aiguë induit par la fièvre ou l'exercice physique, après exclusion des anomalies métaboliques plus communes. Ce diagnostic entraine des conséquences en terme de conseil génétique, du fait du risque potentiel d'hyperthermie maligne peranesthésique.

Mot(s) Clé : Rhabdomyolyse, RYR 1, hyperthermie maligne

N°13#FR8 - Déficit en glycogénine responsable d'une myopathie avec surcharge en polyglucosans : à propos de 2 nouvelles familles.

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La synthèse du glycogène est catalysée par la glycogène synthase et nécessite la présence de la protéine glycogénine. A ce jour, un seul cas décrit présentait une déplétion complète en glycogène au niveau musculaire et une surcharge en glycogène au niveau cardiaque, responsable d'une myopathie associée à des troubles du rythme cardiaque. Nous rapportons ici deux nouvelles mutations responsables d'un déficit en glycogénine-1 identifiées chez trois patients. Cliniquement, nos patients ont une présentation phénotypique sensiblement différente du premier cas rapporté. Un homme âgé de 50 ans présente une myopathie scapulo-péroniére ayant débuté vers 28 ans, sans atteinte cardiaque, avec accumulation de glycogène de structure anormale dans les fibres musculaires. Deux frères jumeaux âgés de 27 ans présentent une myopathie proximale des membres inférieurs, sans atteinte cardiaque. Deux nouvelles mutations à l'état hétérozygote composite ont été identifiées chez le premier patient dont une mutation non-sens (exon 6) et une mutation intronique du site donneur d'épissage (intron 2-3). Cette dernière a été retrouvée chez les jumeaux à l'état homozygote. Ces résultats suggèrent que les mutations du gène GYG1 peuvent se manifester soit par une myopathie scapulopéroniére soit par une myopathie proximale sans atteinte cardiaque.

Mot(s) Clé : Glycogénine, myopathie, glycogène

N°15#FR1 - Manifestations ophtalmologiques dans les myopathies métaboliques génétiques : revue de la littérature

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Afin de recenser les manifestations ophtalmologiques dans les myopathies métaboliques génétiques, une revue bibliographique a été réalisée. La sélection des publications est basée sur une recherche dans PubMed, avec couplage de mots-clés : « ocular diseases » ET « nom de la myopathie métabolique ».
- pour les glycogénoses : maladie de Pompe; maladie de Cori, maladie d'Andersen ; maladie de McArdle ; maladie de Tarui.
- pour les maladies mitochondriales : ophtalmoplégie externe progressive, syndromes MERFF, MELAS, MNGIE.
- pour les lipidoses : déficit en carnitine, en CPT 2, en Acyl CoA déshydrogénase.
La recherche a donné une trentaine de références. Les manifestations ophtalmologiques dans les myopathies métaboliques génétiques sont multiples, mais rarement révélatrices de la maladie. Elles font partie essentiellement des signes d'accompagnement. Certaines myopathies métaboliques ont cependant une expression ophtalmologique originale et spécifique.

Les myopathies métaboliques génétiques justifient une collaboration étroite entre le neurologue des Centres de Référence/Compétence des Maladies neuromusculaires et l'ophtalmologiste afin de mettre en place une prise en charge adaptée. L'examen ophtalmologique est indispensable car des traitements pour limiter le handicap visuel peuvent être proposés. Certains signes oculaires, parfois discrets, nécessitent d'être reconnus par l'ophtalmologiste comme indicateurs d'une myopathie métabolique.

Mot(s) Clé : manifestations ophtalmologiques, myopathies métaboliques, revue bibliographique

N°16#GB9 - A new muscle glycogen storage disease associated with Glycogenin-1 deficiency

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We describe a slowly progressive myopathy in seven unrelated adult patients with storage of polyglucosan in muscle fibers. Genetic investigation revealed homozygous or compound heterozygous deleterious variants in the glycogenin-1 gene (GYG1). Most patients showed depletion of glycogenin-1 in skeletal muscle whereas one showed presence of glycogenin-1 lacking the C-terminal that normally binds glycogen synthase. Our results indicate that either depletion of glycogenin-1 or impaired interaction with glycogen synthase underlies this new form of glycogen storage disease that differs from a previously reported patient with GYG1 mutations who showed profound glycogen depletion in skeletal muscle and accumulation of glycogenin-1.

Mot(s) Clé : Glycogen storage disease, Polyglucosan body myopathy

N°17#FR10 - Accouchement par voie naturelle chez une patiente atteinte de maladie de pompe

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L'accouchement chez les patientes atteintes de la maladie de Pompe doit faire l'objet d'un suivi étroit, notamment sur le plan respiratoire chez les celles présentant une atteinte diaphragmatique. Dans la littérature, l'accouchement par césarienne programmée est recommandé. Nous rapportons dans ce travail les modalités d'accouchement par voie naturelle, chez une patiente âgée de 29 ans atteinte de maladie de Pompe. L'enzymothérapie substitutive (Myozyme) avait été instauré à l'âge de 23 ans. A 32 semaines d'aménorrhée (SA) la patiente a présenté une aggravation de son atteinte respiratoire, motivant la mise en route d'une ventilation nocturne non invasive (VNI). L'accouchement a été déclenché à 34 SA en raison d'une rupture de la poche des eaux, et a été effectué sous anesthésie péridurale par voie basse. Il n'y a pas eu de complication maternelle ou fœtale. La VNI a pu être interrompue 20 jours après l'accouchement, avec retour de la CV à son état de base. Cette observation montre qu'il est possible de réaliser un accouchement par les voies naturelles chez les patientes atteintes de maladie de Pompe, malgré une insuffisance respiratoire sévère. Les conditions requises sont une coordination étroite entre myologue, obstétricien, et pneumologue tout au long de la grossesse.

N°18#FR12 - Aspect particulier de neuropathie périphérique (« ganglionopathie ») dans le déficit en acyl-coA deshydrogénase à chaînes longues (LCHAD)

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INTRODUCTION: Le déficit en Acyl-CoA deshydrogénase à chaînes longues (LCHAD), activité dépendant de l'enzyme mitochondriale trifonctionnelle, est un trouble rare de la bêta-oxydation des acides gras. La forme tardive associe des crises de rhabdomyolyse à une neuropathie périphérique typiquement axonale, sensitivomotrice, longueur dépendante. Nous rapportons deux cas de déficit en LCHAD compliqués d'une neuronopathie sensitive. CASE REPORT : Patient 1: Une femme de 47 ans présentait des épisodes de rhabdomyolyses depuis l'enfance. A l'âge adulte sont apparus des chutes, une maladresse des mains et des paresthésies des extrémités. L'examen clinique révélait une ataxie proprioceptive sévère, une hypoesthésie tactile distale, une aréflexie, des rétractions achilléennes modérées et des pieds creux. L'électroneuromyogramme concluait à une « ganglionopathie ». Patient 2: Une femme de 25 ans souffrait d'une rétinopathie pigmentaire et de rhabdomyolyses récidivantes depuis l'enfance. Elle rapportait également des dysesthésies des membres

inférieurs. L'examen clinique objectivait une hypopallesthésie distale, une aréflexie et des rétractions achilléennes. L'électroneuromyogramme confirmait la neuronopathie sensitive. Dans les 2 cas, le diagnostic de déficit en LCHAD reposait sur le profil des acylcarnitines (élévation de C16-OH et des autres acylcarnitines à longues chaines) et l'analyse génétique (mutations du gène HADHA). CONCLUSION: Le déficit en LCHAD doit être considéré comme une nouvelle étiologie métabolique de « ganglionopathie ».

Mot(s) Clé : Déficit en acyl-coA deshydrogénase à longues chaines (LCHAD), Ganglionopathie, Rhabdomyolyse

N°19#FR19 - Pronostic cardiaque à long terme chez 263 patients atteints de maladies mitochondriales

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Contexte. L'association des maladies mitochondriales à une cardiomyopathie a été rapportée dans de nombreuses séries de patients de faible effectif.

Objectif. Etudier la prévalence d'anomalies cardiaques au sein d'une large cohorte de patients atteints de maladie mitochondriale, étudier le pronostic cardiaque à long-terme et identifier des facteurs pronostiques.

Méthodes. Nous avons inclus les 263 patients avec une maladie mitochondriale prouvée génétique pris en charge dans nos centres entre janvier 2000 et mai 2014. Les informations cliniques et génétiques relatives à leur prise en charge initiales et à leur suivi ont été recueillies.

Résultats. Parmi les 263 patients inclus (age médian=43 ans, hommes=103), 109 avaient une délétions uniques de grande taille de l'ADNm, 64 la mutation m.3243A>G, 53 d'autres mutations ponctuelles de l'ADNm et 37 des mutations de l'ADN nucléaire. Une atteinte cardiaque était initialement présente chez 80 patients : 33 avec des troubles conductifs, 15 une pré-excitation, 17 une hyperexcitabilité ventriculaire, 6 une fibrillation atriale, 48 une cardiomyopathie hypertrophique et 3 une cardiomyopathie dilatée.

Au cours du suivi, 27 patients (10%) ont présenté un événement cardiaque grave. Les deux sous-groupes les plus à risque étaient les patients porteurs de délétions simples avec troubles conductifs et de la mutation m.3243A>G avec hypertrophie ventriculaire gauche. Nous avons identifié les 4 facteurs pronostiques suivants : diabète, trouble de la conduction intraventriculaire, hypertrophie ventriculaire gauche et extrasystolie ventriculaire.

Conclusion. Les patients atteints de maladies mitochondriales ont un risque élevé de développer une complication cardiaque grave.

Mot(s) Clé : maladies mitochondriales, cardiomyopathie

N°20#FR29 - Oxygénation musculaire dans la FSHD

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Objectif: observer les modifications d'oxygénation musculaire d'effort chez des sujets atteints de FSHD
Patients et méthodes : 8 sujets atteints de FSHD et 15 sujets sains ont effectué un effort contrôlé isocinétique d'intensité constante identique à tous les sujets et à 20% de leur moment de force maximal pendant 4 min. L'effort était une succession de flexions/extensions de genou, à la vitesse de 90°/s la flexion étant passive et l'extension d'intensité

contrôlée. Les variations d'oxygénation musculaires étaient mesurées au moyen de la spectroscopie dans le proche infrarourge (NIRS).

Résultats :Les sujets atteints de FSHD développaient un moment de force inférieur aux sujets sains.(-41%) Durant l'exercice, le taux de désoxyhémoglobine (HHb) et le volume sanguin étaient plus bas chez les sujets FSHD. Le moment maximal était corrélé à la fonction de locomotion (test de 6min de marche) et la cinétique de désoxygénation était inversement corrélée à la fonction de locomotion.

Discussion et conclusion : ces résultats suggèrent une altération de la capacité d'extraction de l'oxygène au niveau musculaire qui pourrait témoigner du déconditionnement musculaire.

Mot(s) Clé : Oxygénation musculaire, spectroscopie infrarouge, Dystrophie Facioscapulohumérale

N°21#FR34 - NFATc2 is a major regulator of skeletal muscle myogenesis

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The calcineurin/NFAT (Nuclear Factor of Activated T-cells) signaling pathway plays a regulatory role in skeletal muscle adaptation and muscle regeneration, by its ability to promote myotube differentiation and its role in the establishment of the adult muscle phenotype. Calcineurin dephosphorylates members of NFAT transcription factors allowing NFAT translocation into the nucleus where it cooperates with other transcription factors to induce transcription of target genes. Recently, we demonstrated that NFAT proteins are able to interact with one of the muscle specific transcription factors, MyoD, to control different aspects of skeletal muscle development. The cooperation between the NFATc3 isoform and MyoD is required for primary myogenesis, in the differentiation of somites (Armand et al., 2008).

Similarly, the NFATc2 isoform interacts with MyoD. We found this cooperation to be crucial, as Myod/Nfatc2 double-null mice die at birth, with a dramatic reduction of the major neonatal myosin heavy chain (MHC) isoform normally expressed at birth in skeletal muscles, such as limb and intercostals muscles, whereas its expression is unaffected in myofibers mutated for either factor alone (Daou et al., 2013).

The muscle phenotype of MyoD/NFATc2 double mutant male embryos is also characterized by a marked decrease in dystrophin protein expression at E18.5, compared to wild-type, single mutant and female double mutant littermates at the same age. Furthermore, chromatin immunoprecipitation assays suggest that NFATc2 could be an important regulator of dystrophin expression during myogenesis.

Altogether, these data point out the important role of NFATc2 in regulating different aspects of skeletal myogenesis during mouse embryogenesis.

Mot(s) Clé : calcineurin/NFAT, myogenesis, dystrophin

N°22#FR23 - De novo ceramides synthesis is not involved in skeletal muscle atrophy induced by short-term mechanical unloading

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Patients admitted to the intensive care unit commonly develop skeletal muscle weakness that can exacerbate illness and complicate their recovery. Beyond the primary disease or aging, weakness is promoted by a variety of prolonged hospitalization-associated conditions. These include altered nutritional status, physical inactivity, and prolonged bed rest. The two latter conditions are the most ubiquitous, affecting all patients during a prolonged hospitalization. In both cases, skeletal muscle utilization is decreased with a concomitant reduction in fatty acid oxidation. Subsequent fatty acids accumulation converted to ceramides could be a cellular mechanism leading to muscle wasting. Indeed these sphingolipids act as second messengers in several of molecular signaling pathways involved in muscle atrophy. Consequently, the aim of this work was to determine the effects of immobilization on

muscle ceramide accumulation, and identify the role of these ectopic lipids in molecular mechanisms involved in skeletal muscle atrophy. For this purpose, male Wistar rats were treated with an inhibitor of de novo synthesis of ceramides (i.e. myriocin) and subjected to hindlimb unloading for 7 days. We found that hindlimb unloading increases total muscle ceramide content and decreases soleus muscle weight and fiber diameter. Immobilization increased the level of polyubiquitinated proteins and induced muscle apoptosis. Despite a reduction in total muscle ceramide content, myriocin treatment did not prevent skeletal muscle atrophy and concomitant induction of apoptosis and proteolysis. In conclusion, these results show that de novo synthesis of ceramides is not involved in muscle atrophy induced by a short period of immobilization.

Mot(s) Clé : Sphingolipids, Disuse, Myriocin

N°22bis#GB27 - MUSCULAR PHENOTYPE EXACERBATION OF THE DMSXL MICE, MODEL OF THE MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic neuromuscular disorder due to an unstable (CTG) repeat expansion in the 3'UTR of the DMPK gene. Mutated DMPK mRNA form nuclear foci and affect splicing regulation of various RNA transcripts. The DMSXL mouse model has been created with a large genomic fragment containing the human DMPK gene carrying >1000 CTG. This model mimics molecular, histological defects and most of DM1 phenotype. Nevertheless, as in DM1 patients, the mice's phenotype is variable and sometimes moderate. We aimed at worsening the muscular phenotype in DMSXL mice using forced eccentric exercise (downhill treadmill) to optimize the evaluation of future biotherapies efficacy. Our work suggests that the eccentric exercise can worsen the muscular weakness observed in DMSXL vs. WT, with a significant decrease of their specific maximal force (sP0) in gastrocnemius muscle. That is independently to body weight gain, muscle weight changes or HE staining histological abnormalities suggesting molecular deregulation pathways. Preliminary isoform quantification for candidate genes in WT gastrocnemius revealed that the splicing profile depend on state of development and could be affected for Ldb3 and Mbnl2 mRNA in non-exercised DMSXL vs. WT opening to further molecular investigations in exercised DMSXL.

Mot(s) Clé : Mouse model, Biotherapy

N°23#FR21 - MuSK Frizzled like domain is critical for mammalian neuromuscular junction formation and maintenance

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The muscle specific kinase MuSK is one of the key molecules orchestrating neuromuscular junction (NMJ) formation. MuSK interacts with the Wnt morphogens, through its Frizzled-like domain (cystein-rich domain, CRD). Dysfunction of MuSK CRD in patients has been recently associated with the onset of myasthenia, common neuromuscular disorders mainly characterized by fatigable muscle weakness. However, the physiological role of Wnt/MuSK interaction in NMJ formation and function remains to

be elucidated. Here, we demonstrate that the CRD deletion of MuSK in mice caused profound defects of both muscle prepatterning, the first step of NMJ formation, and synapse differentiation associated with a drastic deficit in acetylcholine receptor (AChR) clusters and excessive growth of motor axons that bypass AChR clusters. Moreover, adult MuSKΔCRD mice developed signs of congenital myasthenia including severe NMJs dismantlement, muscle weakness and fatigability. We also report for the first time, the beneficial effects of lithium chloride, a reversible inhibitor of the glycogen synthase kinase-3, that fully rescued NMJ defects in MuSKΔCRD embryos and therefore constitutes a novel therapeutic reagent for the treatment of neuromuscular disorders linked to Wnt/MuSK signaling pathway deficiency. Taken together, our data reveal that MuSK CRD is critical for NMJ formation and plays an unsuspected role in NMJ maintenance in adulthood.

Mot(s) Clé : jonction neuromusculaire, Wnt signaling, Lithium chloride

N°24#FR25 - Six homeoproteins and myogenic stem cells behavior in mouse fetuses

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Skeletal muscles originate from the paraxial mesoderm, which periodically condensates into somites, from the rostral extremity of the embryo to the tail bud. Muscle progenitors de-epithelialize from the dorsal part of the somite, also called the dermomyotome, to form the future muscle mass. Cells from the ventro-lateral or hypaxial lip of the dermomyotome give rise to muscles of the limb and trunk, and more rostraly to the muscles of the tongue. Cells from the dorso-medial or epaxial lip of the dermomyotome give rise to dorsal muscles.

We have previously shown that the Sine oculis (Six) genes expressed in the myogenic lineage control the expression of Pax3, Myf5, MyoD, MRF4 and Myogenin, all required for skeletal myogenesis. Six1Six4 double mutant mice die at birth, partly because in those mutants, the hypaxial myogenesis is totally abrogated. E18,5 fetuses lack all limb and ventral muscles. Back and cranio-facial muscles form but are smaller in size.

In this study, we compared the characteristics of muscle progenitor cells (Pax7+ cells) in back muscles of wild type and of Six-KO fetuses. We found that the homing of Pax7+ cells in a satellite position is delayed in mutant fetuses and studied the role of Six homeoproteins in the stemness of Pax7+ cells.

Mot(s) Clé : Six1, Satellite cells, Myogenesis

N°25#FR26 - Muscle Satellite Cells loose their functionality during a septic shock: Mesenchymal Stem Cells to the rescue

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Sepsis is an infection resulting in an uncontrolled inflammation leading to tissue damages and organ dysfunctions, causing in 40% of the cases rapid death. Nowadays, treatments are not very efficient and one of the consequences is the loss of muscle mass in half of the survivors (Gamrin et al., 1997; Mittendorfer et al., 1999).
In normal situation skeletal muscle has a great capacity to regenerate ad-integrum after an injury thanks to the muscle stem cells: the satellite cells (SC) (Tajbakhsh, 2003; Zammit et al., 2006; Brack and Rando, 2012; Relaix and Zammit, 2012; Montarras et al., 2013). Having a central role in muscle repair and homeostasis, our work consisted in characterising the behaviour of SC during a septic shock. Indeed although it is known that the initial muscle loss is due to an imbalance between synthesis and degradation of proteins, it does not explain why after 5 years post sepsis the muscle is unable to re-establish its initial mass.

We have shown that after a septic shock SC were not able to repair muscle and that metabolic and mitochondrial functions were impaired for extended periods of time. We have also shown that systemic injections of Mesenchymal Stem Cells were able to decrease the infection as well as repairing the dysfunctional mitochondria by direct transfer to the "non-functional" SC. These results are very promising and could lead to further development in order to better control the inflammation during a sepsis and avoid its long-term negative impact.

Mot(s) Clé : Satellite cells, Mesenchymal stem cells, Sepsis

N°26#FR27 - N-acetylcysteine as an effective treatment in vivo and identification of biomarkers in SEPN1-related myopathy: a first preclinical trial.

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SEPN1-Related Myopathy (SEPN1-RM) is a potentially-lethal congenital muscle disease caused by defects in selenoprotein N (SelN). We showed that SelN is implicated in redox homeostasis, and that the antioxidant N-acetylcysteine (NAC) restores the phenotype in SelN-devoid cultured cells. No treatment is available for SEPN1-RM, its slow progression and the lack of biomarkers hindering implementation of therapeutic trials.

To assess the therapeutic potential of NAC in vivo, determine its dose-effect and identify biomarkers, we performed a preclinical trial with two NAC dosages (NAC20 or NAC60mM) on the sepn1 ko mouse, in sedentary conditions and after chronic forced swimming.

We identified unreported phenotypical abnormalities in the sepn1 ko, including age-related loss of body weight, changes in locomotor activity, reduced half-time to fatigue in exercised isolated muscles, decreased specific muscle force and histological markers of defective adaptative response to exercise. NAC20 restored all these phenotypical outcomes. In contrast, NAC60 had a deleterious impact on several parameters.

Biochemical studies revealed significant alteration of antioxidant enzyme levels, protein carbonylation and oxidized glutathione in muscles and/or in blood samples from sepn1 ko. These biomarkers were restored by NAC20 and/or exercise, without synergistic effect.

In conclusion, NAC is the first effective treatment in vivo in SEPN1-RM models. We establish an optimum dose-effect, report novel measurable outcomes in the sepn1 ko, and identify and validate systemic biomarkers to monitor treatment efficiency on blood samples, thus paving the way for future therapeutic trials.

Mot(s) Clé : SEPN1 related myopathy, Preclinical trial, Antioxidant treatment

N°27#FR31 - Role of Rev-erb alpha in the control of skeletal muscle lipid metabolism

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Skeletal muscle and brown adipose tissue share a common embryonic origin and play key roles in metabolism. The Rev-erb-alpha nuclear receptor is a key regulator of metabolic functions. It is expressed in skeletal muscle and adipose tissue where it controls cell differentiation. However, its role in the control of cell fate decision between skeletal muscle and brown

adipose tissue, and its implication in the control of systemic metabolic homeostasis is poorly understood.

The potential of Rev-erb-alpha to induce adipogenic fate of myogenic cells has been tested by overexpressing Rev-erb-alpha in C2C12 myoblasts, under adipogenic conditions. Results show that Rev-erb-alpha is not sufficient to induce the adipocyte differentiation program in myoblasts but is able to promote lipid droplet formation in myofibers. In addition, our results suggest a role for insulin in this Rev-erb-alpha-dependent intramyocellular lipid accumulation (IMCL).

Our results could therefore bring new insights into the role of Rev-erb-alpha in IMCL accumulation, for which the benefic or harmful role is still controversial. In fact, IMCL accumulation is observed in insulin-resistant obese, Type 2 Diabetes and aging subjects, suggesting a harmful characteristic. However, highly insulin-sensitive and endurance-trained athletes present a similar IMCL content. This is called the "athlete's paradox". Our results therefore suggest a new role of Rev-erb-alpha as a key player of lipid metabolism in skeletal muscle. The use of pharmaceutical agonists for Rev-erb-alpha could offer new therapeutic perspectives to treat muscle and metabolic diseases.

Mot(s) Clé : Rev-erb alpha, Lipid metabolism, Skeletal muscle

N°28#GB6 - Higher sensitivity of myotoxicity detection using a more mature myotube cellular model

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Cerivastatin withdrawal from the market leaded to a considerable economic burden to pharmaceutical industry. Indeed, classical myotube toxicity assays failed to detect statin drugs family as toxicant inducing severe chronic muscle damages and pain. Consequently, pharmaceutical industry needs to develop more relevant in vitro models dedicated to muscle damage drug discovery. In this context, CYTOO developed a physiological human muscle model improving the sensitivity of myotoxic drug detection. Based on our 2D+ technology, primary human myoblasts can form myotubes with high level of striation and nuclei alignment demonstrating a higher differentiation and maturity compared to myotubes formed on standard culture condition. Moreover, 2D+ technology standardizes myotubes formation and enables accessing new readouts for myotubes characterization upon drug treatment. To further demonstrate the benefits of our model, we tested reference drugs inducing hypertrophy or atrophy on both standard culture condition and 2D+ platform. Our results showed that our Muscle Damage model is robust and compatible with High Content Screening. Altogether, the normalization of myotube formation and their higher maturity coupled to enhanced image analysis capacities demonstrated higher predictivity over standard assays which in turn will allow the detection of myotoxic drugs during the early phases of preclinical studies for compound development.

Mot(s) Clé : Myotoxicity detection, Micropatterns,

N°29#FR36 - Analyses of muscle diversification processes by cell specific approaches in Drosophila

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Cell diversification is an essential process for proper organism development. Understanding genetic and molecular mechanisms conferring the individual characteristics of each muscle during development remains a major challenge. For studying muscle diversification process we apply new cell specific approaches and use them in Drosophila model. Indeed, muscle network in Drosophila embryos represents an attractive system for studying cell diversification. It is composed of 30 muscle fibers per hemisegment, which are easy to detect and to follow during development. Currently we take advantage from the newly generated and tested drivers targeting muscle subtypes (Slou, Lms, Duf) and a cell specific transcriptomic method to define a transcriptional

signature that specify differents subsets of muscle cells at differents stages. We first performed translational ribosome affinity purification (TRAP)(Heiman et al, 2008) on Slou positive muscle cells to isolate mRNA engaged in translation. The preliminary results from microarray analyses show enrichment of muscle specific genes. Furthermore biological process gene ontology showed that the majority of enriched genes are linked to muscle development. According to those results, TRAP method seems to be efficient to identify transcriptional signatures in subsets of muscle cells. We are also planning to use chromatin immunoprecipitation after nuclei sorting (INTACT)(Henry et al, 2012) to identify direct targets of identity factor Slou, Lms in muscle subsets. Based on transcriptomic data and ChIPseq at different developmental time points, we will select candidate genes for functional analyses and we will establish their role in muscle diversification processes.

Mot(s) Clé : muscle diversification, gene expression, Drosophila embryo

N°30#GB8 - Gasp-1 overexpression leads to a deregulation of adiposity and glucose homeostasis in adult mice

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Although myostatin is a powerful negative regulator of muscle mass, it also impacts on the metabolism. Indeed, myostatin knock-out (Mstn -/-) mice present an increased muscle mass due to both myofibre hyperplasia and hypertrophy and also an increased insulin sensitivity and a decrease of adiposity preventing them from obesity associated with age. While mice overexpressing Fst or Fstl3, two antagonists of myostatin function, show similar muscle and metabolism phenotypes, overexpression of Gasp-1, an other inhibitor, leads only to a myofibre hypertrophy without loss of fat mass in young adult mice (Monestier et al., 2012; Brun et al., 2014). However Tg(Gasp-1) mice develop age-related obesity associated insulin resistance. To highlight the mechanisms underlying this phenotypic variation, we have undertaken detailed cellular and molecular studies.

Mot(s) Clé : Obesity, Insulin resistance

N°31#GB18 - Altered fatty acid metabolism in mdx mice. Effect of an L-carnitine supplementation.

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As in human Duchenne patients, mdx mice suffer from a mutation in the DMD gene leading to structural defects in muscle cells. Even if a mdx mouse does not share all the phenotypic features of a human patient, these mice are commonly used as a model for the human disease.

We studied alteration in muscle fatty acids metabolism in mdx mice at the age of 14 weeks and we found that (i) the fatty acid composition of the soleus and the gastrocnemius is changed in mdx mice with an increase in PUFA n-6 and a concomitant decrease in the level of MUFA ; (ii) in mdx mice most of the lipogenic activities and some lipolytic pathway enzymes were increased, suggesting an increased turnover of fatty acids in mdx mice. L-carnitine is an amino acid that has been shown to regulate fatty acid metabolism and to interfere with sarcolemma physiology. We supplemented control and mdx mice with L-carnitine and observed a partial restoration of the fatty acid composition parameters in muscles.

Altogether these data suggest that in mdx mice, fatty acid metabolism is altered leading to changes in the composition (and likely the properties) of the membrane of the muscle cells.

Mot(s) Clé : Duchenne mdx, Carnitine

N°32#GB20 - BMP signaling controls satellite cell dependent postnatal muscle growth

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Postnatal muscle growth is achieved by both an increase in myofiber size and the addition of further myonuclei, whereas myofiber number does not increase further. Satellite cells are the resident muscle stem cells which proliferate in growing muscle to supply new myonuclei. Little is known of how satellite cell function is controlled during the postnatal growth phase to permit correct muscle mass development. Here we demonstrate that BMP signaling defines postnatal muscle growth. We found that juvenile satellite cells express P-Smad1/5/8 showing that BMP signaling is active in these cells. Abrogating BMP signaling in satellite cells in juvenile Cre/Lox mice decreased the pool of satellite cells and muscle fibres contained less myonuclei and were smaller than those from control mice. We show that blockade of BMP signaling decreased satellite cell proliferation and diminished the myonuclear recruitment during myofiber growth and this severely retarded muscle growth. In addition, failure of satellite cell proliferation during the postnatal growth phase strongly reduced the final satellite cell reservoir in mature muscle. In conclusion, these results show that correct BMP signaling in satellite cells is required for satellite cell dependent myofiber growth and for the generation of the adult satellite cell pool.

Mot(s) Clé : post-natal muscle growth, satellite cells,

N°33#GB23 - Profiling of circulating miRNAs in response to toxic or traumatic skeletal muscles damages in rats.

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Skeletal muscle damages can occur in a wide range of situations. Circulating miRNAs have been identified as biomarkers of tissue damage. However, whether miRNAs can be used as markers of acute/limited muscle damage is unknown. Our aim was to study plasma miRNAs profiles and kinetics in response to acute muscle injury in rat, to identify new markers.

Muscle injury was induced in a soleus muscle of rats by notexin injection (NTX) or by crushing (CRUSH). Blood was drawn at different time-point post-injury in NTX, CRUSH, sham operated (SHAM) rats and a control group (CTRL) to measure Creatine kinase (CK) activity and circulating miRNAs. A RT-PCR profiling was performed in plasma pools. Seventy nine miRNAs out of 752 were selected based on their expression levels and variations, and were analyzed in individual sample.

Plasma CK increased in NTX 6h and 12h post-injury while no changes significant changes were found in other groups. Plasma levels of muscle-specific miRNAs miR-1-3p, miR-133a, miR-133b, miR-206-3p, miR-208b-3p and miR-499-5p were increased in NTX with a peak value at 12h. The Receiver-Operating Characteristic curves analysis showed a higher diagnostic performance for plasma miRNAs levels as compared to CK activity. Similar profiles were observed for non muscle-specific miR-378a-3p, miR-434-3p and miR-409-3p.

In conclusion, we describe circulating miRNA profiles associated with acute skeletal muscle injuries. Further

analysis will determine how to combine miRNAs measurements to optimize diagnosis.

Mot(s) Clé : Circulating miRNAs, Muscle damage

N°34#FR24 - Exosomes participate in the alteration of muscle homeostasis during lipid-induced insulin-resistance

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Cell-released exosomes can transfer both functional proteins and RNAs between cells. In this study we tested the hypothesis that muscle cells might transmit specific signals during lipid-induced insulin-resistance through the exosomal route. Methods: Exosomes were collected from quadriceps of C57Bl/6 mice fed for 16 weeks with a standard diet (SD) or with SD enriched with 20% Palm oil (HP) and from C2C12 exposed to 0.5 mmol/l palmitate, oleate (EXO-Post Palm and EXO-Post Oleate) or BSA (EXO-Post BSA). Results: HP mice were obese and insulin-resistant. They had altered insulin-induced AKT phosphorylation in skeletal muscle (SKM), reduced Myod1 and Myogenin expressions and increased CyclinD1 mRNA level indicating that palm oil had a deep impact on SkM homeostasis in addition to insulin-resistance. HP mice SkM secreted more exosomes than SD mice SkM. This was reproduced in-vitro using C2C12 cells pre-treated with palmitate the most abundant saturated fatty acid of palm oil. EXO-HP, EXO-Post Palm and EXO-Post oleate induced myoblast proliferation and modified the expressions of genes involved in cell cycle and muscle differentiation but did not alter insulin-induced AKT phosphorylation. Lipidomic analyses showed that exosomes from palmitate-treated cells were enriched in palmitate, indicating that exosomes likely transfer the deleterious effect of palm oil between muscle cells by transferring lipids. Muscle-exosomes were incorporated into various tissues in vivo including the pancreas and liver suggesting that SkM could transfer specific signals through the exosomal route to key metabolic tissues. Conclusions: Exosomes act as 'paracrine-like' signals and modify muscle homeostasis during high-fat diets

Mot(s) Clé : muscle release exosomes, insulin resistance, obesity

N°35#FR18 - Combined IV and ICV scAAV9 and scAAV10-SMNopti delivery dramatically rescues symptomatic SMA mice.

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Spinal Muscular Atrophy (SMA) is a neuromuscular disorder characterized by degeneration of motor neurons (MNs), progressive muscle weakness, and premature death in severe cases. We and others recently demonstrated that intravenous (IV) administration of self-complementary adeno-associated virus vector of serotype 9 (scAAV9), carrying an optimized human SMN1 expression cassette (SMNopti), mediated high and widespread expression of SMN into the MNs, and prevented disease progression in neonatal SMNΔ7 mice (mouse model of SMA).

The objective of this study was to investigate whether scAAV9 or scAAV10-SMNopti delivery could halt or reverse the disease in the severe (Smn-/- ; hSmn2+/+) mouse model of SMA (hSmn2), which born with symptoms.

We first investigated whether the transgene product could be expressed within the therapeutic window in hSmn2 mice (mean survival 4 days) and found that both vectors allowed this protein synthesis. The SMN-expressing vectors were then co-injected into the brain ventricle and the facial vein of post-natal mice

and clinical monitoring of animals was then performed daily. Both vectors post-symptomatic delivery increased survival from 0 to 417 days, representing the highest survival increase obtained to date in hSmn2 mice. High SMN expression levels were found in the brain and spinal cord of the injected mice at final endstage. The neuropathological analyses (MN survival, neuromuscular junction size, muscle atrophy) are in progress.

This study demonstrates the efficacy of SMN-expressing AAV9 and AAV10 for gene therapy of post-symptomatic SMA mice, and highlights the therapeutic potential of the co-IV/ICV route of delivery.

Mot(s) Clé : AAV, post-symptomatic, gene-therapy

N°36#FR5 - Cartographie cutanée de la branche thénarienne du nerf médian

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L'établissement d'une cartographie cutanée de la branche thénarienne du nerf médian permet de l'éviter lors de la libération chirurgicale du canal carpien. Cette étude a été faite à cause des complications opératoires iatrogènes dans le cadre du syndrome du canal carpien, par section des branches nerveuses cutanées palmaire et thénarienne

L' étude anatomique précise le trajet du rameau thénarien du nerf médian par rapport au rétinaculum des fléchisseurs, en particulier en recherchant des branches du nerf médian perforant le rétinaculum des fléchisseurs et atteignant les muscles de l'éminence thénar.

Cette étude a été également faite à cause du problème suivant : Le travail manuel est très important dans les activités professionnelles. Une large proportion d'accidents industriels est représentée par une atteinte partielle ou totale des fonctions de la main. La connaissance de la distribution et des variations des branches musculaires thénariennes du nerf médian est importante dans la mesure où un bilan des lésions nerveuses au niveau de la main est nécessaire pour établir un diagnostic et planifier un traitement chirurgical adéquat.

Une étude en per-opératoire du rameau thénarien du nerf médian a permis de préciser la distribution de ses branches terminales et ceci afin de l'éviter dans la libération chirurgicale du nerf médian dans les cas présentant un syndrome du canal carpien.

Mot(s) Clé : Rameau thénarien, Rétinaculum des fléchisseurs, Syndrome du canal carpien

N°37#FR6 - Etude anatomique du rameau cutané palmaire du nerf médian

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A travers une étude morphométrique et topographique, le travail anatomique réalisé, contribuera à une connaissance de l'origine, du trajet et de la terminaison du rameau cutané palmaire du nerf médian, afin de pouvoir l'éviter lors des abords chirurgicaux et en cas de plaies accidentelles de la face ventrale du poignet.

Cette étude permettra d'avoir une bonne évaluation préopératoire de la région pour la décompression, dans de bonnes conditions, du nerf médian dans le canal carpien. Cette étude a été également réalisée à cause des multiples complications opératoires iatrogènes dans le cadre du traitement du syndrome du canal carpien. La section, par inadvertance, des branches terminales sensitives du RCP au niveau de la région palmaire demeure un risque important.

Mot(s) Clé : Syndrome du canal carpien, Nerf médian, rameau cutané palmaire

N°38#FR11 - Exploration du métabolisme à l'effort et imagerie musculaire dans les formes tardives de déficit multiple en acyl-coA deshydrogénase (MADD)

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INTRODUCTION : La forme tardive de déficit multiple en Acyl-coA deshydrogénase (MADD) est un trouble rare de la bêta-oxydation des acides gras. La biopsie musculaire objective une lipidose, parfois associée à des anomalies mitochondriales. Même traités, certains malades présentent une intolérance à l'effort. Nous rapportons l'étude du métabolisme musculaire in vivo chez ces patients. METHODES : Un test d'effort incrémental sur bicyclette déterminait les capacités à l'effort. La lactatémie était mesurée avant et après exercice. Chaque patient réalisait une IRM musculaire du corps entier et une imagerie fonctionnelle associant spectroscopie au P31, au H1 et imagerie de perfusion. RESULTATS : 5 patients, d'âge médian 35 ans (29-56), atteints d'une forme tardive de MADD ont été inclus. Au test d'effort, la puissance maximale et la capacité aérobie étaient diminuées et le seuil survenait de façon plus précoce chez les patients MADD comparativement aux contrôles. La lactatémie post-exercice était plus élevée chez les patients MADD ayant réalisé un exercice maximal (moyenne = 9.25 mmol/L) que chez les contrôles (7.8 mmol/l). L'IRM musculaire et l'imagerie fonctionnelle étaient normales. CONCLUSION : Dans la forme tardive de MADD, une limitation des capacités d'effort persiste, malgré le traitement. L'hyperlactatémie d'effort témoigne probablement d'un dysfonctionnement mitochondrial secondaire, même en l'absence d'anomalies mitochondriales morphologiques.

Mot(s) Clé : Déficit multiple en acyl-coA deshydrogénase (MADD), Lactatémie, Intolérance à l'effort

N°39#GB14 - Alterations at the cross-bridge level are associated with a paradoxical gain of muscle function in vivo in a mouse model of nemaline myopathy

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Nemaline myopathy is the most common disease entity among non-dystrophic skeletal muscle congenital diseases. The first causative mutation (Met9Arg) was identified in the gene encoding α-tropomyosinslow gene (TPM3). Considering the conflicting findings previously reported for the transgenic (Tg) mice carrying the TPM3Met9Arg mutation, we carefully investigated 8-9 month-old Tg(TPM3)Met9Arg mice on the basis of a multiscale methodological approach. Experiments were performed on skinned muscle fibers and in vivo using nuclear magnetic resonance techniques. While in vitro

maximal force production was reduced in Tg(TPM3)Met9Arg mice, in vivo measurements demonstrated an improved mechanical performance in the transgenic mice as compared to controls. The reduced in vitro muscle force might be related to alterations occurring at the cross-bridges level with muscle-specific underlying mechanisms. In vivo muscle improvement was not associated with any changes in either muscle volume or energy metabolism. Our findings indicate that TPM3(Met9Arg) mutation leads to a mild muscle weakness in vitro related to an alteration at the cross-bridges level and a paradoxical gain of muscle function in vivo. These results point out that in vitro alterations are muscle-dependent and do not translate into similar changes in vivo.

Mot(s) Clé : muscle weakness, Skinned fibers

N°40#GB15 - Quintupling muscle maximal force following mechanical overload in myostatin deficiency

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Muscle mass, fiber type profile and muscle activity are important determinants of muscle force. In our project, we studied the effect of muscle overload, a model for resistance exercise, in two mouse models: wild-type mice and constitutive myostatin knockout mice. Surprisingly, the combination of lack of myostatin and overload resulted in 5 times stronger maximal force generation compared to non-overload wild-type plantaris muscles. Furthermore, overload of mstn-/- plantaris muscle increased the maximal force without further gain in muscle weight and fiber size, resulting in a two-fold increase of the specific force. Whereas wild-type muscle adapted to overload by a conversion towards fast oxidative fiber phenotype. The nuclear domain, which is larger in mstn-/- than in wild-type mice, remained unaffected by overload. Thus, cellular properties of mstn-/- remained unaffected by overload and cannot account for the increased specific force. We hypothesize that extracellular matrix remodeling may be the underlying mechanism responsible for such gain in muscle force production. To approach this question, we will perform transcriptome profiling following muscle overload treatment. In conclusion, our study demonstrates that lack of myostatin increases exercise-induced force adaption, however, the underlying molecular mechanism remains to be elucidated.

Mot(s) Clé : Overload, skeletal muscle

N°41#GB19 - Phosphorylation of NBR1 by GSK3 modulates protein aggregation.

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The autophagy receptor NBR1 (neighbor of BRCA1 gene 1) binds UB/ubiquitin and the autophagosome-conjugated MAP1LC3/LC3 (microtubule-associated protein 1 light chain 3) proteins, thereby ensuring ubiquitinated protein degradation. Numerous neurodegenerative and neuromuscular diseases are associated with inappropriate aggregation of

ubiquitinated proteins and GSK3 (glycogen synthase kinase 3) activity is involved in several of these proteinopathies. Here we show that NBR1 is a substrate of GSK3. NBR1 phosphorylation by GSK3 at Thr586 prevents the aggregation of ubiquitinated proteins and their selective autophagic degradation. Indeed, NBR1 phosphorylation decreases protein aggregation induced by puromycin or by the DES/desmin N342D mutant found in desminopathy patients and stabilizes ubiquitinated proteins. Importantly, decrease of protein aggregates is due to an inhibition of their formation and not to their autophagic degradation as confirmed by data on Atg7 knockout mice. The relevance of NBR1 phosphorylation in human pathology was investigated. Analysis of muscle biopsies of sporadic inclusion body myositis (sIBM) patients revealed a strong decrease of NBR1 phosphorylation in muscles of sIBM patients that directly correlated with the severity of protein aggregation. We propose that phosphorylation of NBR1 by GSK3 modulates the formation of protein aggregates and that this regulation mechanism is defective in a human muscle proteinopathy.

Mot(s) Clé : GSK3, sIBM

N°42#FR28-Mitochondria Dynamics and Muscle Degeneration in C. elegans

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Muscle degeneration is a common feature of aging and muscular pathology such as Duchenne Muscular Dystrophy (DMD). DMD, the most frequent myopathy, is caused by mutations in the dystrophin gene that lead to muscle degeneration in children as seen by muscle weakness and dramatic loss of muscle cells. Moreover, the gradual loss of skeletal muscle mass as an organism ages represents a major factor determining the decline in general health in the elderly population. Currently, the subcellular mechanisms of muscle degeneration remain poorly understood and no curative treatments are available.

Mitochondria play a crucial role in muscle cells in energy production and thus are likely to participate in the cellular processes that lead to muscle degeneration. Our general goal is to determine the role of mitochondria and mitochondria dynamics in the molecular mechanisms leading to muscle degeneration.

Deregulations of mitochondrial network organization are observed in DMD patients. In order to investigate the molecular mechanisms of muscle degeneration, our lab has established a Caenorhabditis elegans mutant DMD model with progressive dystrophin-dependent muscle degeneration: the dys-1(cx18);hlh-1(cc561) double mutant worm. Interestingly, the DMD mutant worm model exhibits increased mitochondrial fragmentation compared to wild type worms suggesting a deregulation in the fusion/fission mitochondrial balance. Furthermore, RNAi mediated knock down of drp-1 - a key protein for mitochondria outer membrane fission - reduces muscle degeneration in C. elegans.

To study the link between mitochondria dynamics and muscle degeneration, we focus on DRP-1 and WAH-1, the worm homologue of Apoptosis-Inducing Factor (AIF).

Mot(s) Clé : mitochondria dynamics, muscle degeneration, C. elegans

N°43#FR30 - Des défauts moléculaires de FAT1 sont associés à une nouvelle pathologie neuromusculaire, la FATopathie

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Identifier les fragments d'ADN responsables des maladies génétiques est plus compliqué que l'amélioration du séquençage avait initialement laissé imaginer. Pour la dystrophie facioscapulohumerale (FSHD), par exemple, des contributions inattendues viennent des modèles de souris portant l'expression défectueuse de la protocadhérine FAT1. Des défauts musculaires et non musculaires imitant la FSH ont été finalement reportés. La FSH de type1 est liée à la contraction du nombre de copies de la séquence macrosatellite D4Z4, de la région subtélomérique 4q35. Le phenotype type-FSHD apparaisse aussi sans contraction. Dans certains de ces cas, des mutations du gène SMCHD1 concomitant à l'hypométhylation de l'ADN en 4q35 permettent de définir l'FSHD de type 2. Cependant, le mécanisme pathologique responsable de FSH reste partiellement élucidé et son diagnostic difficile. Ici, nous reportons des mutations FAT1 chez des patients type-FSHD non porteurs de la contraction D4Z4 ni des mutations SMCHD1 ou du 4q35 hypométhylé. Un épissage anormal du transcrit FAT1 a été prédit pour les mutations retrouvées. Les résultats des tests in vitro pour quatre de cinq mutations sélectionnées montrent des modifications partielles ou totales de l'épissage. L'utilisation d'oligonucléotides antisens a confirmé cette pathogénicité. Les transcrits altérés peuvent produire des protéines FAT1 sans un ou plusieurs domaines extracellulaires de type cadhérine qui peuvent affecter la stabilité ou les interactions avec ses partenaires protéiques. Ces données confirment le rôle de la protéine FAT1 dans la pathogénicité d'une dystrophie type-FSH et définissent une nouvelle entité pathologique qui serait nommé FATopathie.

N°44#FR37 - L'implication de la voie BMP dans le développement musculaire prénatal

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Il a été montré que la voie de signalisation Bone-morphogetic-protein (BMP) est impliquée dans la formation des muscles squelettiques au cours du développement prénatal. Cependant, les principaux mécanismes cellulaires et moléculaires résultants de l'effet de la voie BMP restent inconnus.

Notre objectif est d'expliciter le rôle de la voie BMP dans le développement des muscles des membres. Nous avons croisé des souris Rosa26-Lox-Stop-Lox-Smad6-IRES-EGFP (RS6) avec des souris Lbx1Cre, qui expriment la Cre-recombinase sous contrôle du promoteur du gène Ladybirdhomeobox1 (Lbx1). Lbx1 étant exprimé dans les précurseurs myogéniques, cela permet aux souris RS6+/-Lbx1Cre+/- de surexprimer Smad6 spécifiquement dans les précurseurs myogéniques. Chez les embryons RS6+/-Lbx1Cre+/- à E10,5 et E12,5, nous observons une diminution de l'expression de Pax-3 et de MyoD dans les membres distaux, suggérant un retard du développement musculaire. Au stade E18,5, les fœtus RS6+/-Lbx1Cre+/- ont une diminution du nombre de fibres musculaires comparée aux souris contrôles, certains muscles sont absents et il y a une mauvaise individualisation des muscles. 90% des souris RS6+/-Lbx1Cre+/-meurent peu de temps après leur naissance. Les survivantes ont un retard de croissance, une force spécifique diminuée et un mauvais patterning musculaire. Ces résultats montrent un rôle prépondérant de la signalisation BMP dans la croissance et le patterning musculaire.

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AVANCÉES RÉCENTES DANS LE DOMAINE DES MALADIES MÉTABOLIQUES

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