23 AU 25 NOVEMBRE 2022

ABSTRACT BOOK

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SOCIÉTÉ FRANÇAISE DE MYOLOGIE

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COC01

CLINIQUE

Clinical, morphologic, and molecular features of patients suspected of X-linked myopathy with excessive autophagy.

A. Merlet (1, 2), E. Lacène (3, 4), I. Nelson (5), G. Brochier (3, 4), C. Labasse (6), A. Chanut (6), G. Bassez (7), G. Bonne (5), L. Féasson (1, 8), T. Evangelista (3, 9, 10)

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Contenu

X-linked myopathy with excessive autophagy (XMEA) is a slowly progressive myopathy that affects male patients and is defined by proximal limb weakness. It is caused by mutations in VMA21 gene whose protein product assembles lysosomes' proton pumps. We have studied four patients clinically suspected of XMEA. The clinical charts were reviewed, and extensive histological, immunohistochemical and electron microscopy analysis of the muscle biopsies was performed. Sanger sequencing of VMA21 gene was done. The patients presented typical clinical and pathological features of XMEA with onset during childhood or adulthood, proximal limb weakness and mildly elevated CK. Two patients had cardiac and respiratory abnormalities and one of them had a pacemaker implanted at the age of 43 years-old. Muscle biopsy showed numerous cytoplasmic vacuoles, often multiple within a single fiber, segmented fibers, internalized nuclei and significant variability in fiber size. Vacuoles stained positive for sarcolemmal proteins, LAMP2, LC3, p62 and complement C5b-9. No cytoplasmic aggregates were observed in muscle fibers with antibodies against VCP and heterogeneous nuclear ribonucleoprotein, which exclude multisystem proteinopathies. Ultrastructural evaluation revealed basal lamina duplication, subsarcolemmal and cytoplasmic vacuoles and extensive autophagosome extrusion. Molecular investigation disclosed two pathogenic variants in VMA21 (c.164-7T>G, c.163+4A>G) and a novel variant in the 3′UTR (c*124A>G). No mutation was found for the last patient. In conclusion, we reported a novel mutation and a new clinical aspect with the presence of cardiac abnormalities. Although all muscle biopsies mimic XMEA, we failed to identify VMA21 mutation in one patient suggesting the involvement of additional genes in this unique histopathology. Further studies are needed.

COC02

CLINIQUE

Granulomatous myositis : New insights from a retrospective cohort.

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Objectifs

Granulomatous myositis is a rare entity characterized by symptomatic muscular granuloma formation. This phenomenon is mostly described in sarcoidosis, but can be associated with other diseases or isolated. Currently, four pattern are described in muscular sarcoidosis, associated with different courses of the disease, but prognosis factors remain unknown. We conducted a retrospective study of patients with granulomatous myositis, regarding clinical, biological, anatomopathological and imaging presentations of the disease to identify prognosis factors.

Contenu

Patients were retrospectively included in presence of granuloma in muscular biopsy associated with muscular involvement. Primary outcome corresponded to severe form of the disease defined by Rankin score ≥2 or a progressive form of the disease. Secondary outcomes corresponded to severe motor deficit (MRC ≤3) and difficult-to-treat form, defined by number of therapy lines ≥4. Twenty five patients were studied (14 male) with a median age for muscular symptoms onset at 65 years. Etiology were sarcoidosis (n=15), inclusion body myositis (n=4), auto-immune disease (n=1) and idiopathic (n=5). Distal motor deficit and amyotrophy were significantly associated with a severe form of the disease and severe motor deficit. Regarding treatments, corticosteroids allowed improvement in 75% of cases, but 66% of responders relapses, thus methotrexate appears as a promising second line therapy with no relapse for the responders. We identified an unusual form of the disease called “hypercalcemic” (n=4), characterized by malignant hypercalcemia, granuloma muscular involvement in PET-scan and biopsy, without muscular symptoms. We discuss the presence of anatomopathological criteria of inclusion body myositis within muscular sarcoidosis of our series, evoking an overlap as described in previous studies.

COC03

CLINIQUE

Impact of myotonia on patient quality of life

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Objectifs

Improve understanding of the impact of myotonia in daily life, including mental health, problems with work, studies and socialising, of patients with non-dystrophic myotonia in the French healthcare system.

Contenu

Non-dystrophic myotonias are disabling genetic diseases impacting the quality of life. Due to the coping mechanisms developed by impacted people, improve understanding of the impact of myotonia in daily life is necessary for informative decisions by neuromuscular specialists. That is why we proposed the IMPACT survey, an online patient reported outcome questionnaire completed by 47 non-dystrophic myotonia patients in France. The survey results demonstrate that patients report not only the presence of muscle stiffness (97.9% of patients), but also muscle pain (83.0%), falls (70.2%) and anxiety (76.6%). These difficulties impact their quality of life at work/studies (48.9%) and in a similar proportion the daily life at home or outside. Most of the patients (96%) indicated that they are currently treated with pharmaceutical agent and all of them (100%) reported improvement of muscle stiffness under treatment with 94% cases reporting reduction of falls and 80% reduction of the muscle pain and anxiety. They report an improvement of their quality of life in general due to myotonia treatment. In conclusion, to our knowledge, this is a first and large patient reported outcome survey conducted in France demonstrating the impact of myotonia on patients with non-dystrophic myotonia. It highlights the importance of myotonia management using effective treatments. More work should be initiated to improve patient compliance and treatment adherence in non-dystrophic myotonias.

COC04

CLINIQUE

Caractérisation de l'atteinte faciale dans la myosite à inclusions sporadique et la dystrophie musculaire facio-scapulo-humérale.

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Objectifs

Évaluer la prévalence et les caractéristiques de l'atteinte faciale dans la myosite à inclusions (IBM) et la dystrophie musculaire facio-scapulo-humérale (FSH).

Contenu

Méthodes : 20 patients FSH, 16 patients IBM et 18 témoins sains, ont été inclus prospectivement entre février et août 2022. Tous les participants ont été filmés en réalisant 7 tâches faciales standardisées. Chaque tâche a été cotée par 5 observateurs indépendants de manière semi-quantitative et additionnée pour constituer le Score d'atteinte faciale (SAF). Résultats : Une atteinte faciale, définie ici comme un SAF supérieur à la valeur maximale des témoins était présente chez 10/16 patients IBM (63%) et 18/20 patients FSH (90%). Le SAF était significativement plus élevé chez les patients comparés aux témoins ($p<0,001$) mais il n'existait pas de différence significative entre les patients FSH et IBM ($p=0,313$). Le score d'asymétrie était significativement plus important chez les FSH que chez les IBM ($p=0,001$). La tâche faciale la plus atteinte chez les IBM comparés aux témoins était celle explorant l'orbiculaire des paupières ($p=0,002$) alors que chez les FSH, le déficit de l'orbiculaire des lèvres était significativement plus marqué par rapport aux patients IBM ($p=0,001$). Chez les patients FSH, le SAF était significativement corrélé aux scores cliniques de sévérité et à la durée de la maladie, et chez les patients IBM, à la présence de troubles de la déglutition. Conclusion : Notre étude retrouve une prévalence importante de l'atteinte faciale chez les patients IBM avec des caractéristiques cliniques différentes de celles des patients FSH.

COC05

CLINIQUE

Homozygous COQ7 mutation, a new cause of potentially treatable distal hereditary motor neuropathy

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Objectifs

Distal hereditary motor neuropathy (dHMN) represents a group of motor inherited neuropathies leading to distal weakness. We report a family of two brothers and a sister affected by dHMN in whom a homozygous c.3G>T (p.1Met?) variant was identified in the COQ7 gene. This gene encodes a protein required for Coenzyme Q10 (CoQ10) biosynthesis in mitochondria. This study aims to investigate the pathogenicity of this new variant.

Contenu

We showed that this variation leads to a severe decrease in COQ7 protein levels in the patient's fibroblasts, resulting in a decrease in CoQ10 production, and in the accumulation of 6-demethoxycoenzyme Q10 (6-DMQ), the COQ7 substrate. Interestingly, such accumulation was also found in the patient's plasma. Seahorse experiments showed that the patient's cells mainly rely on glycolysis to maintain sufficient ATP production. Consistently, the replacement of glucose by galactose in the culture medium of these cells reduced their proliferation rate. Interestingly, normal proliferation was restored by CoQ10 supplementation in the culture medium, suggesting a therapeutic avenue for these patients. Altogether, we have identified the first example of recessive dHMN disease caused by a homozygous variation in the COQ7 gene, which should thus be included in the gene panels used to diagnose peripheral inherited neuropathy. Furthermore, 6-DMQ accumulation in the blood can be used to confirm the pathogenic nature of the mutation. Finally, supplementation with CoQ10 or derivatives should be considered to prevent the progression COQ7-related peripheral inherited neuropathy in diagnosed patients.

COC 06

CLINIQUE

MYO-xIA : Quantification de marqueurs pathologiques sur coupes histologiques et exploitation de rapport de biopsie par intelligence artificielle explicative pour le diagnostic de myopathies congénitales.

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Objectifs

L'objectif du projet MYO-xIA est de mieux caractériser les sous-types de myopathies congénitales par l'exploitation automatique par intelligence artificielle explicative (xIA) des coupes et rapports histologiques de patients.

Contenu

Pour exploiter les rapports histologiques de patients, nous avons développé la plateforme IMPatienT qui permet leur numérisation et exploration en se basant sur un système de vocabulaire standardisé pour les différentes méthodes de colorations. À partir des données patients, des visualisations automatiques sont générées et l'ensemble des informations extraites des rapports histologiques (n=89 patients) sont utilisées pour réaliser des suggestions de diagnostic soit par méthodes statistiques (réseau bayésien) ou par xIA à base de règles (LCS). Parallèlement, nous avons développé MyoQuant, une application mettant à disposition des techniques de quantification de marqueurs pathologiques sur coupes histologiques reposant sur les récentes avancées en termes de segmentation de fibres et de noyaux grâce aux réseaux neuronaux. MyoQuant est capable de détecter les noyaux centralisés et périphériques en calculant un score d'excentricité par noyau sur les coupes à la coloration hématoxyline-éosine. Pour la coloration succinate-déshydrogénase mettant en évidence la répartition des mitochondries dans les fibres musculaires, nous avons créé un réseau de neurones quantifiant le nombre de fibres pathologiques selon les répartitions mitochondriales. Ce réseau entrainé sur un total de 16 787 images de fibres musculaires de souris est capable de détecter les répartitions anormales avec une justesse de 92,9 %. MyoQuant et IMPatienT sont disponibles en version de démonstration en ligne : https://lbgi.fr/MyoQuant/ et https://impatient.lbgi.fr/

COF05

FONDAMENTAL

Inhibition of de novo ceramide synthesis promotes skeletal muscle hypertrophy in young mice but does not prevent sarcopenia in old mice

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Objectifs

Sarcopenia is an age-related condition characterized by progressive loss of muscle mass and force. Some studies described an accumulation of bioactive lipids such as ceramides in aged skeletal muscle. Considering that ceramides have been shown to inhibit anabolic signalling, we hypothesized that they could contribute to anabolic resistance and muscle mass loss during aging. The goal of this study was to investigate the role of ceramides and other specific sphingolipids in the pathogenesis of sarcopenia. We aimed to determine if a pharmacological inhibition of de novo ceramides synthesis exerted a geroprotective effect on muscle mass loss

Contenu

We treated adult (7 months) and aged (23 months) mice for 6 weeks with myriocin using subcutaneous osmotic mini-pumps. Surprisingly, the treatment significantly increased the cross-sectional area of gastrocnemius type IIa and IIb/IIx fibers in adult mice. Similarly, myriocin treatment increased C2C12 myotube surface and fusion index. Aged mice exhibited a sarcopenic phenotype with reduced muscle mass and strength that was not prevented or reversed by myriocin treatment. Despite increased surface and fusion index in C2C12 myotubes, treatment did not seem to affect protein synthesis or degradation as quantified by 14C-L-Phenylalanine incorporation and release. Our data demonstrate a positive effect of de novo ceramide synthesis inhibition on muscle fiber size in young mice. The underlying molecular mechanisms are currently being investigated. In perspective, we will investigate whether myriocin can prevent muscle wasting in the context of non-cancerous cachexia.

COF06

FONDAMENTAL

Status and role of PABPN1 nuclear aggregates in Oculopharyngeal Muscular Dystrophy

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Objectifs

Oculopharyngeal muscular dystrophy (OPMD) is a rare genetic muscle disease characterized by an onset of weakness in the pharyngeal and eyelid muscles. The disease is caused by the extension of a polyalanine tract in the Poly(A) Binding Protein Nuclear 1 (PABPN1) protein leading to the formation of intranuclear inclusions (aggregates) in the muscle of OPMD patients. The exact contribution of these aggregates to the human disease is still unclear. On a large collection of human muscle OPMD biopsies we performed an analysis of PABPN1 aggregates in relation with age, genotype and muscle status.

Contenu

We demonstrated that age and genotype influence PABPN1 aggregates. In addition to PRMT1 and HSP70, we identified new components of PABPN1 aggregates including GRP78/BiP, RPL24 and p62. We compated two muscles from the same patient and similar amount of aggregates were observed in different muscles, except for pharyngeal muscle where fewer aggregates were observed. This could be due to the peculiar nature of this muscle which has a low level of PAPBN1 and contains regenerating fibers. To confirm the fate of PABPN1 aggregates in a regenerating muscle, we generated a xenograft model by transplanting human OPMD muscle biopsies into the hindlimb of immunodeficient mouse. Xenografts from subjects with OPMD displayed regeneration of human myofibers and PABPN1 aggregates were rapidly present – although to a lower extent - after muscle fiber regeneration. Our data add supports to the models in OPMD that combine both a toxic role of PABPN1 inclusions and a PABPN1 loss of function.

COF13

FONDAMENTAL

Mutated calcium channel in Exertional Heat Stroke

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Objectifs

To identify genetic causes of EHS and characterize its pathophysiological mechanism.

Contenu

Exertional Heat Stroke (EHS) is a leading cause of sudden death in athletes. EHS occurs in young individuals during strenuous activity and is characterized by hyperthermia and central nervous system dysfunction, from confusion to coma. EHS shares clinical and pathophysiological similarities with malignant hyperthermia (MH), a hypermetabolic crisis triggered by halogenated anesthetics. MH is diagnosed by in vitro contracture test (IVCT). Genetic causes are mutations on RYR1, CACNA1S, or STAC3 genes, responsible for skeletal muscle calcium homeostasis dysregulation. On the opposite to MH, the genetic bases of EHS are still largely unknown. Here we investigated genetic predisposition to EHS by whole exome sequencing on a cohort of soldiers with a positive IVCT. Four unrelated patients were found to harbor genetic variants of the same gene encoding a voltage-gated L-type channel regulatory subunit, that we retained as a major candidate gene for EHS. Thus, we studied first the transcript skeletal muscle expression and identified a new muscle-specific transcript by 3' and 5' RACE-PCR. We also characterized protein expression by western blot and RT-Q-PCR. We performed functional studies after ectopic expression on the cell line model. showed the impact of the mutations on surface targeting by cell surface biotinylation and immunofluorescence. We also explored the interaction of the mutant regulatory protein with other subunits by immunoprecipitation. We currently developed a knock-in mouse model carrying one of the mutations identified, whose phenotypical characterization is ongoing. To conclude, our data, although preliminary, indicate already that mutations alter the physiological channel voltage-gated L-type channel properties.

COF14

FONDAMENTAL

Dlk1-Dio3 miRNA cluster regulates mitochondrial oxidative phosphorylation in Duchenne Muscular Dystrophy

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Objectifs

Investigation of the function of DLK1-DIO3 miRNA cluster in Duchenne muscular dystrophy

Contenu

Duchenne Muscular Dystrophy (DMD) is a severe muscle disease, caused by impaired expression of dystrophin. While mitochondrial dysfunction has an important role in DMD, its mechanism remains elusive. Here we demonstrate that, in DMD and other muscular dystrophies, a large number of Dlk1-Dio3 clustered miRNAs (the largest miRNA cluster of the mammalian genome) are coordinately upregulated in regenerating myofibers and in the serum. To characterize the biological consequence of this dysregulation, 14 DLK1-Dio3 miRNA (DD-miRNAs) were simultaneously overexpressed, in vivo, in mouse muscle. Transcriptomic analysis revealed highly similar changes between the muscle which ectopically overexpressing 14 DD-miRNAs, and the mdx diaphragm muscle with its naturally upregulated DD-miRNAs. Among the commonly dysregulated pathway we found repressed mitochondrial metabolism, of which most strongly, repressed oxidative phosphorylation (OxPhos). Knocking down the DD-miRNAs in iPS-derived skeletal myotubes resulted in increased OxPhos activities. The data suggest that (1) DD-miRNAs are important mediators of dystrophic changes in DMD muscle, (2) mitochondrial metabolism and OxPhos in particular are targeted in DMD by coordinately upregulated DD-miRNAs. These findings provide fresh insight into the molecular mechanism of mitochondrial adaptation in Duchenne muscular dystrophy.

COF17

FONDAMENTAL

Mechanical forces in striated muscles cells

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Objectifs

Mechanical forces are intrinsic to skeletal muscles cells to allow body movements but the cell itself has to deal with the stress induced by the resulting cyclic deformations. We have identified in the past the link between the cytoskeleton and the nucleus in muscle cells, especially Nesprin-1, and its importance in nuclear positioning.

Contenu

It suggests that nuclei in striated muscles are under continuous mechanical stress. This is true in hearts where the absence of LMNA results in cardiomyocytes nuclei with aberrant shape, fibrosis and early death of mice. By removing Nesprin-1 from cardiomyocytes nuclear envelope, we rescue nuclei phenotype, fibrosis and mice live much longer, thus suggesting that diminishing the forces applied to the nucleus in laminopathies can be a therapeutical strategy. We have also developed a platform to study contractile properties at the single myotube level. By combining micropatterning, microscuplture and imaging, we are able to obtain multiple and nearly identical 3D myotubes, 150um long, suspended between pairs of pillars, from a low number of cells. We expect this platform to be a valuable tool in studying contractile and differentiation properties of myotubes coming from low number of patients' cells.

FONDAMENTAL

Combination of antisense oligonucleotide therapy with BIO101 demonstrates synergistic beneficial effects in severe SMA-like mice

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Objectifs

Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by motor neuron loss and progressive muscular atrophy, due to insufficient level of survival of motor neuron protein (SMN). SMA is a non-cell autonomous disease, involving numerous tissues and cell-types, including skeletal muscles. Strategies to overexpress SMN protein and approaches to maintain neuromuscular function are currently developed and appear as promising long-term prospect for SMA therapy. In this context, we evaluated the efficacy of BIO101, a MAS receptor activator, as monotherapy and in combination with ASO therapy in severe SMA-like mice (SmnΔ7/Δ7;tgSMN2+/-).

Contenu

We showed beneficial effects of BIO101 as monotherapy on the entire motor unit with a protection of lumbar motor neurons, a limited muscular atrophy (-17% in the tibialis, -40% in the plantaris, and -12% in the soleus), an increased vascularization (+10% in the tibialis and the plantaris, +3% in the soleus), and an accelerated maturation of both muscular fibers and neuromuscular junctions. These benefits were independent of SMN expression. In combination with ASO therapy, BIO101 demonstrated synergistic effects on body weight (+1% with BIO101, +11% with ASO, +32% with ASO + BIO101 at 10 post-natal days) and increased survival (+5 days of median survival). Importantly, co-treated SMA-like mice improved their moving capacity (+40%) and their muscle fatigue resistance (4,2-fold) compared to SMA-like mice treated only with ASO. These results provide strong evidences that BIO101 constitutes an efficient SMN-expression-independent therapy for improving muscle function and should be considered as a combinatorial option for a new therapeutic strategy in SMA patients.

FONDAMENTAL

The Circadian clock regulates inflammatory process during skeletal muscle regeneration

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Objectifs

Determine the role of the circadian clock on immune cells during regeneration

Contenu

Skeletal muscle homeostasis is ensured by its remarkable ability to control many of its physiological parameters such as its metabolic function or its mass according to the needs of the organism. This tissue has an important capacity to regenerate following injuries caused by intensive exercises or myopathies. Skeletal muscle regeneration requires a well-orchestrated spatio-temporal interaction between satellite cells (SCs) and immune cells, which provides the optimal microenvironment for SC proliferation and differentiation. Circadian rhythms control various physiological functions such as metabolism and immunity. The clock integrates signals related to energy state and regulates many metabolic pathways gating them to the most relevant time of the day. Concerning immunity, the major role of the clock is to coordinate leucocyte circulation and function allowing the body to anticipate phases of the day with higher risk of infections. In this context, we are interested in the role of the circadian clock on the regenerative capacity of skeletal muscle. We have shown that environmental and genetic clock disruption lead to defective skeletal muscle regeneration associated with an alteration of immune cells recruitment, mainly myeloid cells. Furthermore, regenerative process defects observed in our myeloid cells-specific genetic clock disruption models bring out the importance of a functional clock in these cells to control skeletal muscle repair. Ex-vivo co-culture experiments between macrophages isolated from injured muscle and myoblasts have highlighted the central role of macrophages circadian clock in the communication between these cells. Single-nuclei RNA-sequencing have evidenced clock-regulated ligands secreted by macrophages potentially involved in this communication.

FONDAMENTAL

Characterization of the D4Z4 subtelomeric region of a human derived isogenic iPSC line and identification of a CRISPR/Cas9 strategy for DUX4 inactivation in FacioScapuloHumeral muscular Dystrophy type 1 (FSHD1)

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Objectifs

FSHD1 is the second most common muscular dystrophy in adulthood. The defect involves a contraction and a relaxation of the D4Z4 macrosatellite of chromosome 4q35 to less than 11 Repeated Units (RU) associated with the permissive haplotype 4qA161 leading to the myotoxic DUX4 primate specific gene expression. The absence of the D4Z4 region is not associated with the disease. hiPSCs are a good model to comprehend the mechanisms leading to muscular degeneration in FSHD1. The CRISPR/Cas9 system is a promising tool to modify the D4Z4 macrosatellite region altered in FSHD1 patients. The aim of this study is to characterize the D4Z4 macrosatellite region of an isogenic hiPSC line and to design a CRISPR/Cas9 gene editing strategy to reduce the D4Z4 RU number and completely ablate the D4Z4 locus.

Contenu

Haplotype determination was performed by a PCR-sequencing experiment on the 4q35 alleles of interest. A double restriction enzyme digestion coupled to a Southern Blot was performed and validated by Molecular Combing to discriminate and quantify the D4Z4 RU in the 4q35/10q26 alleles. Subsequent sequencing in 10 patients allowed the determination of conserved sequences located upstream and downstream of the D4Z4 RU in the alleles of interest and the identification of undesired SNPs, thus permitting the design of specific SgRNAs for CRISPR/Cas9 experiments. In conclusion, we identified a permissive 4qA161 allele containing 30 D4Z4 RU allowing a FSHD1 modeling thanks to CRISPR/Cas9 approaches. Furthermore, we designed specific SgRNAs suitable for all the tested patients, a significant step to a promising gene therapy.

P01

CLINIQUE

Myotilinopathie : description du phénotype clinique, électromyographique, radiologique et histologique dans une cohorte de 12 patients du sud-ouest de la France

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Objectifs

L'objectif est de décrire le phénotype clinique, radiologique et histologique d'une cohorte de 12 patients suivis pour une myotilinopathie et de comparer nos résultats avec les données de la littérature.

Contenu

Introduction : Les myotilinopathies sont des myopathies génétiques rares dont le phénotype anatomoclinique est hétérogène. L'IRM musculaire permet de dégager un pattern radiologique qui peut orienter le diagnostic.
 Méthodes : Nous avons réalisé une étude observationnelle descriptive rétrospective multicentrique, analysant les caractéristiques cliniques, radiologiques et histologiques d'une cohorte de 12 patients suivis dans les centres de référence des pathologies neuromusculaires de Toulouse et de Bordeaux pour une myotilinopathie.
 Résultats : Dans notre cohorte, 11 patients sont porteurs du variant p. Ser60Cys et 1 patient est porteur du variant p. Ser55Phe. Le phénotype clinique initial est hétérogène, mais l'évolution se fait irrémédiablement vers une atteinte distale ou proximo-distale avec une prédominance distale des membres inférieurs. A partir des 8 IRM musculaires analysées, nous distinguons un pattern radiologique concordant avec les données de la littérature montrant une atteinte des muscles de la loge postérieure (soléaire, gastrocnémien médial) et antérieure (tibial antérieur, fibulaires) des jambes, des muscles de la loge antérieure (vaste intermédiaire, médial) et postérieure des cuisses, des muscles petit et moyen fessier et du grand adducteur, contrastant avec une préservation des muscles droit fémoral et à moindre degré du semi-tendineux, du gracile et du sartorius.
 Conclusion : La description de notre cohorte permet d'enrichir les connaissances sur les myotilinopathies. Ces données phénotypiques sont indispensables à l'analyse de la pathogénicité des variants identifiés par les nouvelles techniques d'analyse génétique.

P04

CLINIQUE

Différents profils cognitifs dans la dystrophie myotonique de type 1

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Objectifs

La dystrophie myotonique de type 1 (DM1, maladie de Steinert) est la myopathie la plus fréquente de l'adulte, causée par une expansion de triplets CTG dans le gène DMPK. L'atteinte cognitive est fréquente, mais semble hétérogène. Nous avons fait l'hypothèse que cette hétérogénéité s'explique par l'existence de profils cognitifs différents.

Contenu

Methodes: 99 patients DM1 ont eu un bilan neuropsychologique au CHU de Lille, évaluant différentes fonctions cognitives. Un algorithme de clustering a été utilisés sur 9 scores cognitifs représentatifs du fonctionnement cognitif global. Résultats: Nous avons observé 3 clusters cognitifs, qui pourraient expliquer l'hétérogénéité observée précédemment. Le premier cluster incluait des patients avec une fonctionnement cognitif plus préservé ; le second cluster montrait de moins bonnes performances executives et attentionnelles ; et le troisième des moins bonnes performances dans tous les domaines. L'âge et le niveau scolaire étaient significativement différents entre les clusters, le cluster avec l'atteinte cognitive la plus sévère étant composé de patients plus âgés et un moins bon niveau scolaire. Il n'y avait pas de différence entre les clusters concernant l'âge de début de la DM1 ni concernant le nombre de triplets. Conclusion: Nous confirmons l'existence de profils cognitifs différents dans la dystrophie myotonique de type 1. Les patients les plus âgés appartenaient au cluster le plus atteint cognitivement, ce qui pourrait indiquer une atteinte cognitive progressive, donc éventuellement un processus dégénératif. Cette dernière hypothèse serait cohérente avec les dépôts de protéine TAU dans le cerveau des patients DM1.

P11

FONDAMENTAL

Macrophage-derived RNAseT2 stimulates muscle stem cell fusion and myofiber formation

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Objectifs

Contenu

During skeletal muscle regeneration, macrophages provide signals that coordinate myogenesis. Recovery macrophages support the final stages of myogenesis, which are differentiation and fusion. Using a screening approach, we identified ribonuclease T2 (RNAseT2) as secreted by anti-inflammatory macrophages. RNAseT2 is a highly conserved secreted factor with a variety of biological properties. RNAseT2 did not impact the differentiation of myogenic cells, but specifically stimulated their fusion. Gain and loss of function in human macrophages, tested in coculture with myogenic cells, confirmed the specificity of RNAseT2 action on cell fusion. We showed that RNAseT2 enters myogenic cells via the mannose receptor, which is required for myogenic cell fusion. Actin cytoskeleton remodeling is required for myogenic cell fusion. Our results showed an increase in actin stress bundle formation in myoblasts treated with recRNAseT2. Treated myoblasts showed differential expression of genes involved in actin remodeling, confirming the role of RNAseT2 in actin bundle assembly. In vivo gain-of-function experiments, using plasmid electroporation, validated the profusogenic effect of RNAseT2 during skeletal muscle regeneration, assessed by an increase in the number of myonuclei in regenerating myofibers. Immunoprecipitation and mass spectrometry experiments identified Ste20-like protein kinase (SLK) as a partner of RNAseT2. In the absence of SLK, the effect of recRNAseT2 on fusion was abolished in vitro. Furthermore, Proximity Ligation Assay (PLA) experiments showed colocalization of SLK with recRNAseT2 in myoblasts. In conclusion, our results show that recovery macrophages support the myogenesis fusion step, by secreting RNAseT2, that, in complex with SLK, controls actin reorganization in the fusing myoblasts.

P17 / PM3

CANDIDAT AU PRIX MASTER

FONDAMENTAL

Role des lamines A/C dans la mise en place et/ou le maintien des marque epigenetiques lors de la differenciation et de la reponse aux dommages de l’ ADN dans les laminopathies des muscles stries

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Contenu

Le gène *LMNA* code pour les lamines A/C, des filaments intermédiaires situés sous la membrane intérieure du noyau. Les lamines A/C sont impliquées dans différentes fonctions cellulaires comme l’organisation de la chromatine et la réparation de l’ADN. Les mutations de ce gène causent des maladies rassemblées sous le nom de laminopathies ; parmi elles se trouvent les laminopathies des muscles striés qui sont les plus nombreuses. Nous avons pu montrer que dans cette pathologie les cellules musculaires étaient plus sensibles à l’induction de dommages de l’ADN et que leur système de réparation des dommages serait affecté. Cependant il ne semble pas y avoir de défaut d’import de protéines de réparation de l’ADN, comme 53BP1, contrairement à d’autres laminopathies. Le système de réparation des dommages de l’ADN est surement affecté à un autre niveau qui reste encore à découvrir.

P21

CLINIQUE

Molecular mechanisms of metabolic perturbations in DMD

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Objectifs

Based on improved understanding of the pathophysiology, we aimed at the development of combined therapy for Duchenne muscular dystrophy.

Contenu

The cloning of the dystrophin gene during the late 1980 early 1990's was followed by a structure-function analysis, which provided the basis for the "mechanical stabilization" hypothesis. Accordingly, a critical function of dystrophin is the mechanical stabilization of the myofiber and cardiomyocyte. Large amount of experimental data supporting this hypothesis, which is also in agreement with the phenotype in DMD. However, accumulating data suggests that dystrophin fulfills additional functions. Moreover, some observations in DMD are inconsistence with the mechanical stabilization hypothesis solely. We profiled miRNA in the plasma in a cohort of DMD patients [1]. These dysregulated miRNAs were subdivided into distinct families, some of which with indications for involvement in metabolic functions [2]. We demonstrated that dysregulated miRNAs of the DLK1-DIO3 locus are associated with mitochondrial functions [3–5]. We found that the overall pattern of miRNA dysregulation is consisted with perturbations of lipid metabolism [1]. Our data support that metabolic perturbations play important role in DMD. A progress in the understanding of these metabolic perturbations promotes the development of combined therapy approaches for DMD [6]. 1. Amor et al. J. Cachexia. Sarcopenia Muscle 2021, 12, 677–693, doi:10.1002/jcsm.12708. 2. Israeli et al. Non-coding RNA 2022, 8, 48, doi:10.3390/NCRNA8040048. 3. Sanson et al. Sci. Rep. 2020, 10, 9139, doi:10.1038/s41598-020-66016-7. 4. Vu Hong et al. Eur. J. Transl. Myol. 2021, 31, 2021, doi:10.4081/ejtm.2021.10012. 5. Vu Hong, et al. bioRxiv 2021, 2021.10.20.464950, doi:10.1101/2021.10.20.464950. 6. Bourg et al. Int. J. Mol. Sci. 2022, doi:10.3390/IJMS23042016.

P24

CLINIQUE

Study of PABPN1 regulation in human skeletal muscle

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Objectifs

OPMD is a rare muscle genetic disease mainly characterized by ptosis and dysphagia. The disease is caused by a short (GCN)1-8 expansion in the polyadenylate RNA binding protein nuclear 1 (PABPN1) gene. The mutation leads to the formation of intranuclear aggregates in the muscle of OPMD patients. Despite numerous studies stressing the deleterious role of nuclear inclusions, their exact contribution to the disease is still unclear. PABPN1 is an ubiquitous protein and its major role is the activation of the poly(A) polymerase (PAP). Loss of function experiments have shown that decreasing levels of PABPN1 leads to defects in myogenesis, muscle atrophy and altered RNA metabolism. In human and mouse, PABPN1 protein level is low in skeletal muscle, as compared to other organs. During mouse muscle regeneration, PABPN1 expression is high in muscle stem cells and myoblasts but low in mature skeletal muscle. Today little is known about the mechanisms that regulate and control PABPN1 expression in human skeletal muscle.

Contenu

In this context, we are analyzing the regulation of PABPN1 in human skeletal muscle with the final aim to restore a functional PABPN1 level in muscle. We are generating and validating a CRISPR-cas9 cell line with reduced level of PABPN1 expression to investigate the effect of PABPN1 depletion in human muscle cells as well as tools and expression vectors of known PABPN1 regulators (HuR and circPABPN1) and potential other PABPN1 regulators identified by bioinformatic analysis. By the end of this project, we should be in a position to understand how PABPN1 is regulated in muscle and to propose means of upregulating PABPN1 in muscle.

P25

CLINIQUE

Genetic background swapping questions the contribution of mutated Ltbp4 in atrophy and fibrosis of the D2.mdx murine model of Duchenne muscular dystrophy

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Objectifs

The original mdx mouse (C57BL/10ScSn background) and mdx5Cv mouse (C57BL/6J background) are widely used as models for Duchenne muscular dystrophy (DMD). However, they show a mild disease, compensatory muscle hypertrophy, late-onset fibrosis, and normal lifespan. Recently, the D2.mdx mouse emerged as an attractive model for DMD: transferring the mdx allele into the DBA/2J background caused frailty, muscle atrophy and enhanced fibrosis. This enhanced phenotype would be due to a mutation in Ltbp4 (Ltbp4DEL), a polymorphism associated with poor prognosis in DMD. The DBA/2J background also has a mutation in Abcc6 (Abcc6MUT), responsible for ectopic calcification in dystrophic muscle cells, a feature not relevant to DMD. To decipher the contribution of Ltbp4DEL and Abcc6MUT in aggravating the phenotype of dystrophic mice we generated new mouse lines by transferring Ltbp4DEL and/or Abcc6MUT to the C57BL/6J background. The four resulting lines were characterized up to 12 months of age and compared to mdx5Cv and wildtype (WT) mice.

Contenu

At all ages examined mice bearing Ltbp4DEL and/or Abcc6MUT had similar body weight as WT and mdx5Cv mice. Muscle size and isometric force was similar in all groups of dystrophic mice, independently of Ltbp4DEL and Abcc6MUT alleles. As anticipated, Abcc6MUT caused ectopic calcification in muscles but did not induce muscular atrophy and weakness. Fibrosis was similar in all groups, suggesting that, in the C57BL/6J background, Ltbp4DEL does not promote fibrosis and atrophy. Our findings question the roles of Ltbp4DEL in aggravating the phenotype of dystrophic mice and strongly suggest that other genetic factors are involved.

P27

CLINIQUE

Identification of CTG repeat contraction factors in myotonic dystrophy type 1

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Objectifs

Myotonic dystrophy type 1 (DM1) is a neuromuscular disease caused by an unstable CTG repeat expansion in the DMPK gene. Larger expansions are associated with more severe symptoms and a decreasing age of onset. We hypothesize that the development of innovative therapeutic strategies, aimed at decreasing the CTG repeat length, and thus to stop or reverse the progression of the disease, may improve the quality of life of patients. The specific objective of our work is to uncover bioactive molecules able to induce repeat contractions in trinucleotide repeat (TNR) models and to decipher the mechanisms promoting these contractions using efficient tools.

Contenu

We performed a large-scale screen for pharmacologically relevant chemical modulators of instability using the Prestwick Library taking advantage of a chromosomal GFP reporter that can accurately measure CTG repeat changes in a HEK293 cell population. The effect of selected molecules directly on the dynamics of CTG repeat instability is studied in HEK293 cells as well as DM1 fibroblasts using targeted long-read sequencing. During the chemical screen, we identified candidate molecules notably involved in epigenetic regulation pathways, that may modulate the size of CTG repeats. Some of these molecules induced stabilization or even contractions of CTG repeats in the HEK cell model and in DM1 fibroblasts. The direct perspective of our work is to identify new small molecules and new druggable targets promoting CAG.CTG repeat contractions, thus offering new therapeutic perspectives for DM1 and TNR diseases.

P29

FONDAMENTAL

Enhancing the differentiation potential of human iPSC-derived myogenic progenitors for the development of a high-throughput screening cellular platform

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Objectifs

Based on the expertise of our laboratory, our objectives are: (1) to optimize and standardize myogenic differentiation protocol of hiPSC, (2) to improve the fusion capacity of hiPSC-derived myogenic progenitors (3) to develop a 3D human muscle model by combining hiPSC-derived myogenic progenitors and biomimetic hydrogels developed in our laboratory.

Contenu

Rapid advances in gene therapy and drug discovery for the treatment of neuromuscular disorders (NMD) have exacerbated the need to develop standardized and miniaturized cellular platforms reproducing the structural and functional characteristics of native skeletal muscle. Accordingly, important progress has been made in the field of skeletal muscle engineering, notably by moving from 2D models to more sophisticated 3D models. For this purpose, human induced pluripotent stem cells (hiPSC) are of great interest, since they can be easily amplified, give access to cells carrying various genetic mutations and allow the engineering of isogenic models. However, protocols for myogenic differentiation of hiPSC remain largely suboptimal. We have recently established a new protocol based on the use of finely controlled media that significantly improves the differentiation of hiPSC into myogenic progenitors (up to 100% of myogenic cells). However, these myogenic progenitors are immature and display limited fusion capacity in 2D conditions. Interestingly, by differentiating these progenitors on soft hydrogels mimicking the biochemical and biomechanical cues of the skeletal muscle ECM, we could greatly improve their fusion and obtain well aligned myofibers. Therefore, using this strategy we aim at modeling different neuromuscular disorders (such as Duchenne Muscular Dystrophy) to provide high-throughput screening cellular platforms.

P32

CLINIQUE

Identification of Novel Genes for Congenital Myopathies

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Objectifs

Congenital myopathies (CM) are rare genetic muscle diseases present at birth or early infancy. They are characterized by hypotonia and muscle weakness associated with structural anomalies of the muscle fibers. Approximately half of the patients do not have a genetic diagnosis, precluding the understanding of the pathomechanisms and the development of therapies. In order to identify causative CM genes, we recruited more than 300 families of patients.

Contenu

Based on the clinical presentations of the patients and histopathological features on the muscle biopsies, we established 5 homogeneous cohorts: 14% of the cases were classified as central core disease (CCD), 17% were classified as CM with protein aggregates (PAM), 27% as centronuclear myopathy (CNM), 21% as rare myopathies (RM) and the last 21% as undefined myopathies. Overall, more than 700 patients and healthy relatives underwent on Next Generation Sequencing . The data were analyzed and filtered using an in-house bioinformatic pipeline. The subgroup analyses led to genetic diagnosis for 56% of CNM families, 40% of CCD, PAM and RM families and 23% of undefined myopathy families. Three cases were highlighted: mutations in genes previously linked to the phenotype, mutations in known genes with a different phenotype, mutations in novel genes. Currently, genetic investigations and functional characterization of candidate genes are ongoing for the undiagnosed families. The identification of novel CM genes and mutations will improve molecular diagnosis and ameliorate disease management. The investigation of the implicated pathways will contribute to a better understanding of the underlying pathomechanisms and may uncover therapeutic targets.

P40

CLINIQUE

Evènements inattendus de l'analyse RNAseq du gène COL6A1 chez un patient avec Dystrophie Musculaire Congénitale d'Ullrich

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Objectifs

Les myopathies liées au collagène VI (COLVI) (Myopathie de Bethlem et dystrophie musculaire congénitale d'Ullrich) sont caractérisées par une atteinte musculaire, une hyperlaxité articulaire, des rétractions et une atteinte respiratoire progressive. Le diagnostic est basé sur les signes cliniques, l'IRM musculaire, l'Immunofluorescence de COLVI sur fibroblastes, et le séquençage NGS d'un panel de gènes permettant l'identification de variant(s) dans les gènes COL6A1-A2-A3. L'approche transcriptomique par RNAseq nous a permis de mieux évaluer l'impact de ces variants sur l'expression du COL6.

Contenu

Un jeune garçon est adressé pour une faiblesse musculaire, une hyperlaxité distale et des chutes fréquentes, avec CK normales. L'analyse d'exome a montré la présence de deux variants dans COL6A1 : c.717+4A>G hérité paternel et c.1003G>A, p.(Gly335Arg) hérité maternel. Les parents sont asymptomatiques et leurs IRM musculaires sont normales. Le variant c.717+4A>G est rapporté pathogène associé à une transmission récessive. Le variant c.1003G>A, p.(Gly335Arg) touche le premier nucléotide de l'exon 14, une Glycine de la triple hélice non décrite dans LOVD, et de fréquence allélique non rapportée dans GnomAD. L'analyse par immunofluorescence du COLVI dans les fibroblastes montre une expression avec absence de sécrétion et rétention intracellulaire du COLVI. L'analyse du transcriptome par RNAseq de COL6A1 a permis (1) d'identifier les conséquences potentielles de ces deux variants en terme de pattern d'épissage et (2) d'évaluer la quantité d'allèles mutés. Les résultats combinés de l'analyse des transcrits de COL6A1 et de celle de l'expression et de la sécrétion du COLVI au niveau des fibroblastes seront discutés.

P44

CLINIQUE

Signe de Beevor et myopathie : et si ce n'était pas une FSH ? A propos d'un cas.

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Objectifs

Le signe de Beevor est très spécifique de la myopathie FascioScapuloHumérale (FSH), il n'est cependant pas pathognomonique. Nous rapportons le cas d'un patient ayant un diagnostic différentiel.

Contenu

Il s'agit d'un patient de 25 ans sans antécédent personnel ou familial. Vers l'âge de 20 ans, il développe progressivement des difficultés à monter les escaliers, des troubles de la marche, quelques myalgies d'effort, sans plainte aux membres supérieurs. Cliniquement, marche très dandinante avec hyperlordose et steppage bilatéral. Il existe un franc signe de Beevor. Aux membres inférieurs, déficit proximal et distal assez symétrique côté à 2-3/5 (MRC), excepté des quadriceps parfaitement conservés. Aux membres supérieurs, scapula alata bilatérale très modérée et discrètement asymétrique, déficit moteur proximal à 4/5 environ, corde du brachio-radialis moins bien perçue à gauche. Aucune atteinte faciale, ni pli pectoral. Les CPK sont à 1,5-2 fois la normale. L'ENMG retrouve des tracés myogènes avec quelques activités de repos anormales. Au plan génétique, la région D4Z4 est normale avec ≥ 11 UR, non en faveur d'une FSH. Le panel de dystrophie des ceintures retrouve une mutation hétérozygote composite du gène GNE. Dans une série de patients avec myopathie GNE, le signe de Beevor est présent dans 15/17 (=88%) cas. Le signe de Beevor reste très spécifique d'une myopathie FSH, mais lorsque des atypies pour une FSH sont notées (déficit trop symétrique, absence d'atteinte faciale ou de scapula alatae notamment), il est quand même possible d'évoquer d'autres myopathies, notamment celles associées à des mutations GNE.

P50

CLINIQUE

Impairment of myogenesis by interferon mediated epigenetic remodeling in inflammatory myopathies

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Objectifs

Inflammatory myopathies (IM) are a group of auto-immune diseases that are all characterised by severe muscle impairment. In spite of the use of immunosuppressive drugs, a significant proportion of patients show residual muscle weakness. IM-derived muscle stem-cells (MuSC) have impaired proliferation and myogenesis in vitro. This intrinsic dysregulation seems to be linked to IFN-signaling. Indeed, IM patients are characterised by a strong IFN signature, and inhibition of IFN-signaling rescued their myogenic properties. On the other hand, IFN-stimulation of healthy patients-derived MuSC mimicked the proliferation defect of IM-MuSC.
 Interestingly, it has been shown that the histone chaperone HIRA upon IFN stimulation is relocated to PML-nuclear bodies (PML-NB) and in parallel to IFN-stimulated genes (ISG). Deletion of HIRA during myogenesis in vitro and in vivo has also been shown to inhibit the expression of muscle regulatory genes and impairs muscle regeneration.
 Our hypothesis is that abnormal IFN stimulation of MuSC in IM patients induces loss of HIRA in myogenic genes and its relocalization to ISG. This would leads to persisting impairment of muscle regeneration and sustained inflammation. Investigation of the link between IFN, myogenesis and HIRA-PML might uncover a novel epigenetic signalization that regulate muscle regeneration dysregulation in IM.

Contenu

We are investigating the impact of IFN-stimulation before or after induction of differentiation on MuSC derived from healthy patients. We asses their ability to differentiate, as well as their transcriptome and epigenome using immunofluorescence, RT-qPCR and chromatin immunoprecipitation.

P52

CLINIQUE

Revisiting systemic sclerosis-associated muscle involvement in light of automated morphometry

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Objectifs

To reevaluate the myopathological features of systemic sclerosis-associated muscle involvement

Contenu

Systemic sclerosis (SSc) is characterized by interstitial fibrosis and microangiopathy. SSc patients with muscular involvement are usually classified in 'SSc myositis', which is characterized by inflammation and myonecrosis, or 'SSc myopathy', characterized by fibrosis, microangiopathy and atrophy of type II muscle fibers. We retrospectively analyzed the myopathological features from 66 SSc patients and 15 control patients. FIJI program and home-made algorithms were used for quantifying these features. SSc patients were classified as SSc myopathy (52%), myositis (30%) and necrotizing autoimmune myopathy (NAM; 18%). Compared to controls, SSc showed a higher percentage of total connective tissue area (CTA) (31% vs 23%, p=0.03), especially myopathy patients (p=0.017). Endomysial capillaries were fewer in number (p=0.029) and occupied a larger area (p<0.001). Myositis type associated with increased size and decreased number of capillaries (p<0.001 and <0.01, respectively), while the two other types had enlarged capillaries (p=0.02 and 0.003, respectively). Cross-section surface area (CSA) of type II myofibers was reduced in all types and atrophy score was the highest in myositis group. Finally, whatever the type, the different parameters (CTA, microangiopathy, type II fibers atrophy) correlated between them. In conclusion, fibrosis, microangiopathy and type II fibers atrophy constitute the hallmarks of muscle changes specifically induced by SSc, which may associate or not with myositis or NAM. Combining the quantified histological features with clinical parameters will help to more accurately stratify patients and more appropriately tailor the therapeutic strategy.

P66

CLINIQUE

Tolérance et efficacité à long terme de l'efgartigimod chez des patients atteints de myasthénie généralisée : Résultats intermédiaires de l'étude ADAPT+

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Objectifs

L'efgartigimod a démontré une amélioration cliniquement significative (ACS) vs placebo dans l'étude ADAPT chez des patients atteints de myasthénie autoimmune. Les patients ayant terminé l'étude ADAPT étaient éligibles pour l'étude d'extension ouverte de 3 ans, ADAPT+.

Contenu

L'efgartigimod 10 mg/kg a été administré par voie intraveineuse en cycle(s) de perfusion hebdomadaire pendant 4 semaines. Les cycles suivants étant initiés en fonction de critères cliniques prédéfinis. 91% des patients de l'étude ADAPT (151/167) ont été inclus dans ADAPT+. En février 2021, 106 patients RACh+ et 33 RACh- avaient reçu ≥1 dose d'efgartigimod (dont 66 patients sous placebo provenant d'ADAPT). La durée moyenne de l'étude était de 363 (114) jours, soit 138 patients-années -d'observation. Des taux similaires d'EI ont été observés dans les bras efgartigimod-efgartigimod et placebo-efgartigimod: céphalées (15,1%/30,3%), rhinopharyngite (8,2%/13,6%), diarrhée (6,8%/10,6%). Cinq décès sont survenus; aucun n'a été considéré comme lié à efgartigimod par l'investigateur. Les EI étaient principalement d'intensité légère ou modérée. Une ACS a été observée chez les patients anti-RACh+ au cours de chaque cycle à des degrés comparables aux améliorations à la semaine 3 du cycle 1 (moyenne: MG-ADL, –5,1[0,34] ; QMG, –4,7[0,41]). Les améliorations cliniques ont reflété les réductions maximales des IgG totales et des anti-RACh dans tous les cycles. Des résultats similaires ont été observés chez les patients RACh–. Ces résultats suggèrent que l'efgartigimod au long cours est bien toléré et efficace chez les patients atteints de myasthénie autoimmune suivis à 1 an dans l'étude d'extension ouverte ADAPT+.

P68

CLINIQUE

Besoin accru en ressources médicales et sociétales des patients atteints de myasthénie modérée à sévère par rapport à la population générale : analyse des données observationnelles digitales de 8 pays

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Objectifs

Comparer la productivité au travail et l'utilisation des ressources médicales (URM) des patients atteints de myasthénie (MG) modérée à sévère (msMG) avec la population générale.

Contenu

L'étude MyRealWorld-MG est une étude numérique, observationnelle, internationale menée chez des patients adultes atteints de MG. Les patients atteints de msMG ont été identifiés comme ceux ayant un score total MG-ADL > 6. L'étude observationnelle POPUP (Standards chez la Population Générale) a recueilli des données similaires et a été menée dans plusieurs pays. L'étude POPUP a inclus des sujets parmi le grand public et les échantillons nationaux étaient représentatifs en termes d'âge, de sexe, d'éducation et de région. Les données POPUP ont été appariées à l'étude MyRealWorld-MG. L'étude POPUP a inclus 9 000 sujets, l'étude MyRealWorld-MG - 880 patients ayant rempli l'échelle MG-ADL; parmi eux, 431 avaient msMG. 57,1% des patients msMG avaient besoin d'un soignant, contre 7,1% POPUP. De plus, 43,5 % des patients msMG ont eu un arrêt de travail au cours du mois précédent (durée de 14,8 jours, ET 12,0), quatre fois plus élevé que dans POPUP (10,2 %, durée de 13,1 jours, ET 11,6). L'URM a révélé que le taux d'hospitalisations était multiplié par un facteur 17, le nombre de visites aux urgences par 10 et le nombre de visites chez un spécialiste par mois était multiplié par 4 pour la msMG par rapport à l'étude POPUP. La msMG est associée à des coûts médicaux et sociétaux plus élevés.

P70

CLINIQUE

Rozanolixizumab in generalized Myasthenia Gravis: Responder Analyses From the Phase 3 MycarinG Study

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Objectifs

Generalized myasthenia gravis (gMG) is a rare neuromuscular disease caused by pathogenic immunoglobulin G (IgG) autoantibodies. Rozanolixizumab inhibits the neonatal Fc receptor, reducing serum IgG, including pathogenic IgG. The objective is to evaluate the efficacy and safety of rozanolixizumab in patients with gMG.

Contenu

The double-blind, Phase 3 MycarinG study (MG0003/NCT03971422) recruited 200 patients (≥18 years, AChR+ or MuSK+ gMG, MGFA Class II–IVa), randomizing them 1:1:1 to weekly subcutaneous rozanolixizumab 7mg/kg, 10mg/kg or placebo for 6 weeks. The primary endpoint was change from baseline (CFB) at Day 43 in MG Activities of Daily Living (MG-ADL) score. Responder endpoints at Day 43 were MG-ADL (≥2.0-point improvement), Quantitative Myasthenia Gravis (QMG) and Myasthenia Gravis Composite (MGC) (both ≥3.0-point improvement). Additional endpoints included Minimal Symptom Expression (MSE, MG-ADL score of 0 or 1) and safety. Patients were randomized to rozanolixizumab 7mg/kg (n=66), 10mg/kg (n=67) or placebo (n=67). Day 43 least squares mean CFBs in MG-ADL (difference vs placebo [95% CI]) were: −3.370 (−2.586 [−4.091, −1.249]) for 7mg/kg; −3.403 (−2.619 [−3.994, −1.163]) for 10mg/kg; and −0.784 for placebo ($p<0.001$ for both doses versus placebo). More patients in the rozanolixizumab 7mg/kg and 10mg/kg arms than the placebo arm achieved response in MG-ADL (71.9%, 69.4% and 31.3%), QMG (54.7%, 72.6% and 39.1%) and MGC (60.9%, 74.2% and 40.6%). MSE was achieved in 25.8%, 28.4%, and 3.0%. Treatment-emergent adverse events occurred in 81.3%, 82.6%, and 67.2%. Efficacy of rozanolixizumab was supported by higher responder rates than placebo. Rozanolixizumab had an acceptable safety profile and was generally well tolerated. Fundings: UCB Pharma.

CANDIDAT AU PRIX MASTER

FONDAMENTAL

The myofiber intrinsic role of AMPKα2 in the regulation of myonuclear accretion

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Objectifs

Contenu

Muscle stem cells (MuSCs) are essential for skeletal muscle homeostasis and contribute to this through activation, proliferation, differentiation, and subsequent fusion. They would fuse with a pre-existing myofiber to increase its nuclei number. This process is called myonuclear accretion and contributes to muscle regeneration, hypertrophy, and maintenance throughout life. MuSCs are localized below the basal lamina and share their microenvironment with myofibers. Since MuSCs fate is determined by metabolic changes in their microenvironment, metabolic variations in myofibers could influence it to promote myonuclear accretion. As AMPK is a main regulator of metabolism, we hypothesized that it regulates the metabolic communication between myofibers and MuSCs. We focused on the muscle isoform AMPKα2 within myofibers during myonuclear accretion. Myonuclear accretion was induced in vivo through neuromuscular electrical stimulation (NMES) on a murine model where AMPKα2 was only deleted in myofibers. With an EdU-based method, new added myonuclei were counted as myonuclear accretion index. The number of nuclei per fiber was higher in the muscles subjected to NMES, but this increase did not differ between wildtype and AMPKα2 deleted mice. In vitro, myofibers treated with AMPK activators and differentiated MuSCs derived from primary MuSCs were cocultured. The number of EdU+ nuclei in myofibers seemed to increase when AMPK was activated by the compound 991 and the metformin only in myofibers. These data suggest that AMPKα2 is not required for the basal regulation of myonuclear accretion, but its role through long term adaptations of metabolism needs further investigations.

P79

FONDAMENTAL

Expression of channel proteins in human skeletal muscle in response to 5 days of dry immersion

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Objectifs

Skeletal muscle deconditioning (loss of muscle mass and function) is one of the consequences of spaceflight that strongly affects astronauts. Here, we determined the expression of channel proteins involved in water mineral balance and excitation-contraction coupling in skeletal muscle of sixteen male volunteers before and after 5 days of DI, an Earth-based model of simulated microgravity.

Contenu

Skeletal muscle deconditioning was characterized by a decrease in maximal voluntary contraction (-12% P<0.01) and thigh cross-sectional area (-2% P<0.01), together with molecular evidence of a reduction in myofibrillar gene expression and repression of translation. DI was associated with important changes in the expression of channel proteins as shown by decreased water channel aquaporin 4 (AQP4) mRNA (-15%, P=0.05) and protein level (-25%, P<0.05), decreased plasma membrane Ca2+ ATPase (ATP2B1) (-19%, P<0.05) and sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2 (ATP2A2) (-75%, P<0.01) mRNA level and increased protein level of Na+/K+ ATPase a2 (ATP1A2) (50%, P<0.05). We also evidenced an increase in acetylcholine receptor a1 (CHRNA1) transcript level (P<0.05). No variation was observed for the transcript levels of CLC1 (Cl- transporter), SCN4A (Na+ voltage gated channel), CACNA1S (Ca2+ voltage gated channel), RYR1 (Ca2+ release channel), ATP2A1 (sarcoplasmic/endoplasmic reticulum Ca2+ transporting 1), ATP1A1 (Na+/K+ channel), NCX (Na+/Ca2+ exchanger), TRPC1 (nonspecific cation channel). Together, these data suggest that 5 days of simulated microgravity remodels the expression profile of critical channel proteins in skeletal muscle tissue, which may impact the capacity of skeletal muscle to generate force.

P81

FONDAMENTAL

Chronic activation of ALK5/TGFbRI signaling in adult mouse skeletal muscle induces severe muscle wasting with concomitant impaired mitochondrial integrity

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Objectifs

Background Transforming Growth Factor β (TGFβ) pathway is a major negative regulator of skeletal muscle mass. Dysregulation of TGFβ signaling is increasingly being implicated in muscle wasting in chronic diseases (myopathies, cancer...) and aging sarcopenia. However, the impact of chronic TGFβ activation restricted to skeletal muscle has not yet been examined. Methods We have generated a new conditional mouse model to activate TGFβ signaling in adult myofibers through the muscle-specific and inducible expression of a constitutively active ALK5/TGFβRI receptor, also called TGFβRI-CA (RCA). The pathophysiology of dysregulated TGFβ signaling in skeletal muscle was investigated.

Contenu

Results We observed that expression of a constitutively active ALK5 receptor in adult myofibers promoted activation of Smad2/3 signaling leading to severe muscle wasting, fiber type shift and progressive reduction in muscle force. Reduced myofiber size is induced by decreased protein synthesis, upregulation of FoxO transcription factors activity and activation of Ubiquitin-Proteasome-System catabolic pathway. Interestingly, changes in Akt/FoxO signaling underlie the muscle remodeling overtime in these mice. Our results moreover identified impaired autophagy flux, associated with a progressive accumulation of abnormal mitochondria and impaired mitochondrial respiration upon chronic activation of ALK5 signaling in muscle. Conclusions Our study provides the first transgenic mouse model to investigate the impact of cell-autonomous, inducible and chronic activation of TGFβ signaling in skeletal muscle. Altogether, our data show that chronic activation of ALK5 signaling in adult muscle fibers leads to severe muscle wasting and concomitant loss of mitochondrial function.

P83

FONDAMENTAL

Neuromuscular electrical stimulation training induces myonuclear accretion and hypertrophy in mouse without overt signs of muscle damage

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Objectifs

Contenu

Skeletal muscle is a plastic tissue that adapts to increased mechanical loading/contractile activity through fusion of muscle stem cells (MuSCs) with myofibers, a physiological process referred to as myonuclear accretion. However, it is still unclear whether myonuclear accretion is driven by increased mechanical loading per se, or occurs, at least in part, in response to exercise-induced muscle injury. Here, we developed a non-damaging protocol to evaluate contractile activity-induced myonuclear accretion/hypertrophy in a physiological context. On that basis, contractile activity was generated by applying repeated electrical stimuli over the mouse plantar flexor muscles and was carefully monitored in response to each stimulation train and for each trained mouse. This method is commonly referred as to NeuroMuscular Electrical Simulation (NMES) in Human and was performed under isometric conditions to avoid muscle damage. NMES training led to a robust myonuclear accretion and higher MuSC content in two mouse lines with a different genetic background, without overt signs of muscle damage/regeneration. We further demonstrated that NMES-induced myonuclear accretion is an early event preceding muscle hypertrophy. This new mouse model of myonuclear accretion that relies on the main function of skeletal muscle, i.e., force production in response to electrical stimuli, would be relevant to further identify the cellular and molecular mechanisms regulating MuSC fate (i.e., from activation to fusion) in overloaded muscle. It would also allow to decipher the role of cell-cell interactions and related secreted factors in a physiological context of muscle hypertrophy.

P85

FONDAMENTAL

Caractérisation de la sous-population d'ASC provenant du tissu adipeux et impliquée dans la régénération musculaire

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Objectifs

Caractériser la ou les sous-populations d'ASC provenant du tissu adipeux sous-cutané et qui migrent dans le muscle suite à une lésion musculaire.

Contenu

La dégénérescence musculaire accompagnant le vieillissement est un problème de santé majeur. On la retrouve chez certaines maladies d'origine génétique (Myopathie de Duchene) mais également dans des myopathies acquises (Myosite à inclusion). Dans le muscle, les cellules progénitrices fibro-adipogéniques (FAP) jouent un rôle critique au cours du processus de régénération. En effet, ces cellules mésenchymateuses (MSC) permettent le maintien d'un microenvironnement favorable aux cellules satellites, initient l'adipo-fibrose et interagissent étroitement avec les cellules de l'immunité. Le laboratoire a récemment montré que d'autres MSC, les ASC, provenant du tissu adipeux sous-cutané, sont impliquées dans la régénération du muscle suite à une blessure musculaire. Dans ce travail, nous utilisons une approche scRNA-seq pour caratériser les ASC qui migrent dans le muscle suite à une lésion. Nous avons identifié plusieurs sous-populations candidates dont les fonctions joueraient un rôle pro-régénérant. Notre projet sur le long terme permettra d'élaborer de nouvelles approches de thérapie cellulaire en utilisant les sous-populations ASC pro-régénérantes pour restaurer la régénération musculaire.

P87

FONDAMENTAL

Increased myofiber activity promotes muscle stem cell fusion, reduces inflammation and improves muscle function in a mouse model of cancer cachexia

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Objectifs

Contenu

Cancer cachexia (CC) is characterized by systemic inflammation resulting in drastic body weight loss, skeletal muscle wasting (i.e., reduced muscle size) and weakness (i.e., reduced force production). CC reduces patient survival and no curative treatments exist. Emerging studies reveal that CC may result from dysfunction of muscle stem cells (MuSCs). Indeed, tumor-derived circulating factors block both MuSC differentiation and fusion leading to muscle atrophy. The regulation of myogenesis is also influenced by the dynamic interactions between MuSCs, myofibers and immune cells (i.e., macrophages). We recently demonstrated that increased myofiber contractile activity by neuromuscular electrical stimulation (NMES) promotes MuSCs fusion in healthy muscles. We aimed to determine whether NMES improves MuSC fate and reduces muscle weakness, wasting and inflammation in the mouse model of CC bearing the C26 carcinoma. We showed that the NMES training improves muscle force and mass in C26 mice together with changes in myofiber-type composition. These functional, structural and metabolic changes occur in association with MuSC fusion improvement and transition toward an anti-inflammatory status of macrophages in muscle, validated by both in vivo and in vitro studies. These findings demonstrate that stimulated myofibers positively regulate MuSC fate and tissue inflammation to counteract the deleterious effects of CC.

P90

FONDAMENTAL

GDF5 as rejuvenating treatment for age-related neuromuscular failure

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Objectifs

Sarcopenia is a disease defined as progressive age-related loss of muscle strength, function and mass, which results in increased mortality. Several mechanisms have been proposed to explain the onset and progression of sarcopenia, however, some pathophysiological aspects are still not very well understood and no cure has been established to date. Our previous work demonstrated that GDF5/BMP14 (Growth Differentiation Factor 5/Bone Morphogenetic Protein 14) overexpression in old mouse prevented muscle mass decline, although a deeper report on the mechanisms and consequences of GDF5 implement on aged muscle was missing.

Contenu

Here, we demonstrate that GDF5 overexpression in muscle during aging induces muscle mass gain, improves neuromuscular junction (NMJ) morphology and nerve/muscle connectivity. In addition, we present the characterization of the cellular and molecular effects of GDF5 in muscle during aging and show its "rejuvenating signature". Based on this proof of concept, we defined a cutting-edge therapeutic approach describing how the treatment with the recombinant GDF5 protein (rGDF5) is able to counteract the age-related skeletal muscle wasting in mice and might have a strong curative potential on humans.

P91

FONDAMENTAL

Excitability and Ca2+ handling defects in skeletal muscle fibers from a zebrafish model of Bethlem myopathy

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Objectifs

Bethlem myopathy (BM) is a muscle disease characterized by joint contractures and muscle weakness worsening with age. BM results from mutations in genes encoding one of the three α chains of collagen VI (COLVI), a component of the skeletal muscle extracellular matrix. A still unresolved issue in BM is how alteration in COLVI present outside muscle fibers induces dysfunction within muscle fibers. Data obtained in COLVI-deficient animal models indicate that sarcoplasmic reticulum (SR) structure is altered, suggesting that mutated COLVI in BM may compromise muscle excitability and/or Ca2+ homeostasis.

Contenu

In the present study, we succeeded in implementing the current- and voltage-clamp techniques combined with intracellular Ca2+ measurements on isolated fast skeletal muscle fibers from one-year old zebrafish harboring an exon-skipping mutation (col6a1Δex14) that is the most frequently found in BM patients. Muscle action potentials were found to be unchanged in col6a1Δex14 fish. The voltage-dependence of charge movements of dihydropyridine receptors, that control SR Ca2+ release, was found to be reduced and shifted toward negative potentials in col6a1Δex14 fish. Concomitantly, the voltage-dependence of intracellular Ca2+ transients was shifted toward negative voltages over the whole membrane voltage range, promoting a pathogenic SR Ca2+ leak at resting membrane potentials. Finally, swimming performance was found to be reduced in col6a1Δex14 fish, suggesting that muscle weakness and wasting observed in BM could be caused at least partially by alteration in Ca2+ signaling identified in the present study. Future experiments will consist in testing therapeutic agents targeted to Ca2+ signaling to treat this currently incurable disease.

P92

FONDAMENTAL

Characterization of pathological features in skeletal muscles of acid ceramidase deficient mice and correction by gene therapy

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Objectifs

Farber disease (FD) and spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME) are two lysosomal storage disorders resulting from loss-of-function mutations in the ASAH1 gene encoding for acid ceramidase (ACDase), an enzyme that cleaves bioactive ceramides in fatty acid and sphingosine. Accumulation of ceramides in tissues results in macrophage infiltration and inflammation, with clinical manifestations of various degrees of severity. Although skeletal muscle abnormalities were reported in patients, in particular in SMA-PME patients, which manifest progressive muscle weakness and presence of groups of small atrophic myofibers suggestive of a denervation process, the role of ACDase in muscle is poorly understood.

Contenu

In the present study, we characterized skeletal muscles of Asah1P361R/P361R mice, an animal model of severe acid ceramidase deficiency with reduced life expectancy (7-13 weeks). Histological analyses showed progressive muscle fiber atrophy and infiltration of macrophages. The level of ceramides were quantified in quadriceps of mutant mice at 10 weeks of age by liquid chromatography tandem-mass spectrometry (LC-MS/MS) and found significantly increased, indicating that muscle might be primarily affected in the disease. In addition, we evaluated the effect of intravenous administration of a recombinant AAV9 vector expressing ASAH1 ubiquitously and observed a correction of the muscle phenotype. These data suggest that skeletal muscles should be targeted when developing novel therapies for severe ACDase deficiency.

P93

FONDAMENTAL

Gene therapy strategies using CRISPR/Cas9 for RYR1-related myopathies

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Objectifs

Type 1 ryanodine receptor (RyR1) is the intracellular calcium channel responsible for skeletal muscle contraction. Mutations in RYR1 gene can lead to RyR1-related myopathies (RyR1-RM), a group of genetic disorders. They are characterised by abnormal calcium homeostasis and muscle weakness of variable severity. Currently, there is no treatment for these pathologies. This study aims to present proof of concept for gene therapy approaches.

Contenu

Immortalized skeletal muscle cells from patients affected by RyR1-Related myopathies have been edited using Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9 (CRISPR/Cas9). CRISPR guides targeting different part of the gene were design in order to adapt the strategy depending on the patient's mutation and its localisation. The consequences of the expected editing will be verified at the DNA, RNA and functional level. The objective is to determine if these therapeutic approaches could improve calcium release in patient's cells and skeletal muscle contraction.

P94

FONDAMENTAL

Normalization of DYNAMIN 2 activity through CRISPR/Cas13-mediated mRNA decay as a pan-therapy for Centronuclear myopathies

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Objectifs

Autosomal dominant mutations in DNM2 coding for Dynamin2 (DNM2) cause moderate forms of Centronuclear myopathies (CNM). DNM2 (ubiquitous GTPase) is involved in endocytosis, membrane trafficking and actin cytoskeleton dynamics. In CNMs due to mutations in other genes, elevated DNM2 protein level is found. It has been shown that reduction in DNM2 levels rescues these CNMs phenotype. Our objective is the normalization of DNM2 expression level through CRISPR/Cas13-mediated mRNA decay as a pan-therapy for CNM.

Contenu

We contributed to the characterization of an original DNM2R465W/+ canine model that carries the most common human DNM2 mutation and that parallels the human disease course. Cas13 protein was recently identified for RNA instead of DNA editing, highly efficiently and with less unwanted off-targeting than conventional RNA interference. We used CRISPR/Cas13 specific RNA targeting to diminish DNM2 transcript. As a translational proof of concept, we have set up the technique first on primary myoblasts sampled from muscle biopsies of healthy and DNM2R465W/+ dog littermates. Myogenic potential, autophagy flux, endocytosis, GTPase activity and mitochondrial respiration are being characterized, as a set of quantifiable tools for measure the phenotype improvement after treatment. GTPase activity was found increased in DNM2R465W/+ myoblasts due to the gain-of-function mutation. We confirmed a significant reduction in DNM2 mRNA transcript in CRISPR/Cas13 transfected myoblasts, together with a decrease in the GTPase activity. Once validated in cells, this strategy will be applied to DNM2R465W/+ canine model by intra-muscular injection and phenotype recovery will be studied. Phenotypic improvement and system safety will be assessed in a second time by systemic injection to determine if a translation to the clinic is possible.

P95

FONDAMENTAL

Characterization of a dog model of autosomal dominant centronuclear myopathy carrying the most common DNM2 mutation in patients.

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Objectifs

Gain-of-function mutations in DNM2 lead to autosomal dominant centronuclear myopathy (CNM) characterized in most cases by an onset during adulthood, and mild clinical course. Mutations in other genes causing CNM induce elevated content of DNM2 in muscle. Therapeutic strategies based on reducing the amount of DNM2 or targeting pathogenic mechanisms are emerging. Our objective was to identify and characterize a canine model of DNM2-related CNM, for preclinical trials.

Contenu

In a Border collie male with locomotor disabilities and hallmarks of CNM (muscle biopsy), we identified the c.1393C>T (R465W) mutation in DNM2, the most common in humans. We generated a litter, and provide a full set of data on the five littermates, four carrying the DNM2R465W mutation. Histological abnormalities dominated by abnormal distribution of mitochondrial network, were detected from 2-months and aggravated with age, also including autophagy impairment and centralized nuclei. Dogs showed a markedly decreased muscle force from 4 months of age with a moderately progressive evolution of clinical signs. Locomotion was mildly impaired, characterized by a slightly decreased forward propulsion. Some dogs tended to exhibit slight difficulties to jump and to stand on their pelvic limbs. After 36 months of age, two dogs exhibited reduced jaw opening. From 9 months of age, atrophy of masticatory and paraspinal muscles was observed and confirmed by MRI at 17-months. CK values were moderately increased in two dogs at 3-4 months, and remained normal afterwards. Myostatin was markedly and lastingly decreased from the age of 4 months. These dogs parallel the human disease course and this extensive characterization provides basis for their use as a relevant animal model in preclinical studies.

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FONDAMENTAL

Pharmacological inhibition of HDAC6 downregulates TGF-β via Smad3 acetylation and improves dystrophin-deficient muscles

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Objectifs

The absence of dystrophin in Duchenne muscular dystrophy (DMD) disrupts the dystrophin-associated glycoprotein (DGC) complex resulting in fiber fragility and atrophy, associated with disorganization of microtubules and of the neuromuscular junction (NMJ) as well as to fibrosis. The non-conventional cytoplasmic histone deacetylase 6 (HDAC6) was previously shown to impede acetylcholine receptor distribution and promote muscle atrophy.

Contenu

Here we show that administration of the specific HDAC6 inhibitor tubastatin A to the mdx mouse model for DMD improves muscle strength, restores microtubule, NMJ and DGC organization and protects against muscle atrophy and fibrosis. Unexpectedly, we found that the beneficial effects of HDAC6 inhibition also involve the downregulation of transforming growth factor-beta (TGF-β) signaling by increasing acetylation of the downstream effector Smad3 in the cytoplasm, thereby preventing their phosphorylation, nuclear translocation, and transcriptional activity. These findings provide in vivo evidence that Smad3 is new target of HDAC6 and implicate HDAC6 as a potential therapeutic target in Duchenne muscular dystrophy.

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CANDIDAT AU PRIX MASTER

FONDAMENTAL

Modulation of intracellular pathways involved in the AAV trafficking to optimize AAV-based therapies in Duchenne muscular dystrophy and autosomal dominant Centronuclear Myopathy

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Objectifs

Adeno-associated virus (AAV) serotype 8 is of particular interest as a vector used in gene therapy for neuromuscular disorders, e.g. Duchenne muscular dystrophy (DMD) and X-Linked Myotubular Myopathy (MTM1). However, toxicities have recently emerged with high-dose AAV gene therapies in clinical trials for these myopathies. Therefore, optimization studies are needed to reach maximal therapeutic benefit with the lowest dose of vector. In addition, our studies performed on animal models of DMD and autosomal dominant centronuclear myopathy (CNM) showed that AAV-mediated transgene expression is lower in the pathological muscles compared to healthy controls. In this context, we are studying the mechanisms underlying the AAV-mediated transduction in diseased muscle, especially regarding the intracellular trafficking of the vector which is the rate-limiting step of AAV transduction in many cell types.

Contenu

In this study, we showed that autophagy and endo-lysosomal pathways, two parallel degradative pathways, are disturbed in muscle cells from patients with autosomal dominant CNM and DMD leading to a potential limitation of AAV-based therapies. These results open the way toward development of optimized AAV-mediated transduction through modulation of intracellular pathways in pathological muscles.

P98

FONDAMENTAL

Preclinical assessment of therapeutic cocktails in dystrophic mice: Tamoxifen combined to metformin, citrulline and steroids

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Objectifs

We published earlier that tamoxifen (Tam) had remarkable protective actions in mdx5Cv mice, an animal model of Duchenne muscular dystrophy (DMD). Subsequently, clinical trials have been launched. The British charity Duchenne UK is supporting massively the international Phase 3 clinical trial TAMDMD and wanted to make funding decisions about other drug candidates, including citrulline (Cit) and metformin (Met), compounds believe to improve muscle perfusion and enhance mitochondrial biogenesis. Consequently, Duchenne UK requested that we evaluate the efficacy of Tam+Cit+Met cocktails in dystrophic mice, using a comprehensive panel of functional, biochemical, molecular and histological investigations.

Contenu

Male dystrophic mice were treated for 3 months at 8-9 months of age. First we determined the best combination of Tam+Cit and Tam+Met. The highest doses caused minor toxicity. Next, we combined the 3 drugs at the optimal dosing, with or without prednisolone (Pred) and compared their efficacy to Tam alone, Tam+Pred and Met+Cit. Addition of Cit+Met to Tam marginally increased the efficacy of Tam alone. Overall, addition of Pred to either Tam or Tam+Cit+Met slightly improved motor function and muscle force. Unexpectedly, Cit+Met slightly worsened the condition. Molecular and histological analyses are ongoing. Our findings suggest that (i) Cit+Met is not a valid option for DMD, (ii) the therapeutic benefit of adding Cit+Met to Tam is limited, (iii) Pred slightly enhances the improvements afforded by Tam, (iv) addition of Cit prevents muscle fatigue. Further investigations are warranted to confirm and extend these findings which may have important consequences for developing efficient therapies for DMD patients.

P99

FONDAMENTAL

Development of versatile allele-specific siRNAs able to silence all the dominant dynamin 2 mutations

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Objectifs

Dominant centronuclear myopathy (CNM) is a rare form of congenital myopathy ranging from severe neonatal to milder adult forms and due to heterozygous mutations in the DNM2 gene encoding Dynamin 2 (DNM2). Dominant DNM2 mutations also cause rare forms of Charcot-Marie-Tooth disease and hereditary spastic paraplegia, and deleterious DNM2 overexpression was noticed in several diseases. The proof of concept for therapy by allele-specific RNA interference devoted to silence the mutated mRNA without affecting the normal allele was previously achieved in a mouse model and patient-derived cells, both expressing the most frequent DNM2 mutation in CNM. However, more than 30 DNM2 mutations are associated with in AD-CNM and our objective is to enlarge the number of patients eligible for allele specific therapy.

Contenu

In order to have versatile small interfering RNAs (siRNAs) usable regardless of the mutation, we have developed allele-specific siRNAs against two non-pathogenic single-nucleotide polymorphisms (SNPs) frequently heterozygous in the population. In addition, allele-specific siRNAs against the p.S619L DNM2 mutation, a mutation frequently associated with severe neonatal cases, were developed. The beneficial effects of these new siRNAs are reported for a panel of defects occurring in patient-derived cell lines. The development of these new molecules allows targeting the large majority of the patients harboring mutations or overexpressing DNM2 by only a few siRNAs, and represents an important step for the preclinical development of this therapy.

P100

FONDAMENTAL

Etude rétrospective sur l'utilisation des dispositifs de compensation des membres supérieurs chez 10 patients atteints de maladies neuromusculaires âgés de 8 à 25 ans.

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Objectifs

Evaluer l'intérêt des dispositifs de compensation des membres supérieurs dans la vie quotidienne de patients neuromusculaires âgés de 8 à 25 ans.

Contenu

Les pathologies neuromusculaires peuvent entrainer une faiblesse des membres supérieurs associée à celle des membres inférieurs dès l'âge pédiatrique. Les activités de la vie quotidienne seront alors perturbées et génèrent une dépendance vis-à-vis d'une tierce personne. Une alternative est l'utilisation d'aides techniques compensant la perte de fonction des bras. Avec le soutien de l'AFM téléthon et du Conseil régional AURA, ces dispositifs sont proposés sur la plateforme D'AC-Membre Sup (Dispositif d'aide à l'Acquisition d'une aide technique de Membre Supérieur) au CHU de Clermont-Ferrand. Ces outils peuvent portés sur une activité spécifique (repas), sur la compensation d'un groupe musculaire (support de bras mécanique ou électrique coude-épaule) ou sur la compensation complète du membre supérieur (bras robotisé complet). Notre étude rétrospective évalue l'intérêt des dispositifs dans les activités jugées importantes par les patients, en examinant la fatigabilité, les douleurs au cours de leur utilisation. Les données générales recueillies comportent l'âge, la pathologie, le score MFM-D2 et la force de préhension. Le critère de jugement principal est le score MCRO (Mesure Canadienne du Rendement Occupationnel). Les critères secondaires sont les scores EVA (Echelle Visuelle Analogique), FSS (Fatigue Severity Scale), de Brooke, et l'évaluation de la force musculaire. Les résultats préliminaires sur 6 patients montrent l'importance de l'activité « repas » et la nécessité de proposer précocement ces outils. Dix patients seront inclus dans ce travail et aideront à juger de l'intérêt des dispositifs chez les jeunes patients.

P101

FONDAMENTAL

Improvement of muscle structure and function in a mouse model for BIN1-related centronuclear myopathy following tamoxifen administration

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Objectifs

Contenu

Centronuclear myopathies (CNM) are congenital disorders characterized by muscle weakness and abnormal centralization of nuclei within muscle fibers. The main genes associated with CNM are MTM1, DNM2, BIN1, RYR1 and SPEG. To date, no effective treatment is available. However, an increased survival and improved muscle function was reported in a mouse model for severe MTM1-related CNM after administration of the pharmacological agent tamoxifen. Therefore, we investigated the effects of tamoxifen-enriched diet from 3 to 8-week-old in the Bin1mck-/- mouse model modeling the typical form of BIN1-related CNM. Macroscopic, cellular and molecular phenotypes were assessed and compared with wild-type (WT) littermates. In-situ muscle force production was markedly improved in Bin1mck-/- treated mice after tamoxifen treatment and associated with a slight enhancement in fiber size. Histologically, the mitochondria mispositioning observed in untreated Bin1mck-/- mice was fully reversed in tamoxifen-treated Bin1mck-/- mice. At the protein level, dynamin 2, involved in the CNM disease process, and cullin 3, a marker of ubiquitin-proteasome system (UPS), were increased in untreated Bin1mck-/- mice, and normalized following tamoxifen treatment. Moreover, the overactivation of Akt/mTOR pathway illustrated by the hyperphosphorylation of Akt and S6 in the Bin1mck-/- model was partially prevented by tamoxifen. Overall, our data suggest that tamoxifen antagonizes disease development through the regulation of dynamin 2 level which likely involves modulation of UPS. In conclusion, the positive effects of tamoxifen on muscle phenotype in Bin1mck-/- mice points out that tamoxifen may serve as a therapy for several forms of CNM.