P2.45
Proteomic studies of muscle progenitor cells in affected and non-affected oculopharyngeal muscular dystrophy muscles
Thérapie des maladies du muscle strié, Institut de Myologie, U976 – UPMC Univ. Paris 6/Inserm UMR7215 – CNRS, Paris, France; Service d’Oto-Rhino-Laryngologie et de Chirurgie Cervico-Facial, Faculté de Médecine St Antoine, Université Pierre et Marie Curie, Hôpital Tenon, Paris, France; Genethon, Evry, France; Hillel Yaffe Medical Center, Department of Neurology, Hadera, Israel

Oculopharyngeal muscular dystrophy is an autosomal dominant inherited muscle disorder caused by a GCG repeat amplification in the coding region of the polyA binding protein nuclear 1 gene. Typical features of OPMD include a late onset and a specific involvement of the pharyngeal and cricoaryngeal muscles with progressive eyelid drooping and swallowing difficulties. The main pathological hallmark of the disease is intranuclear aggregates of mutated PABPN1. Although the cause of the disease and the consequences on affected muscles are known, the pathological mechanisms leading to the alteration of the myogenic program remain to be determined. We have previously demonstrated that cell cultures isolated from non-affected OPMD muscles have a normal proliferation and differentiation phenotype, whereas cultures isolated from affected muscles have a reduced myogenicity and proliferative capacity as compared to control. To study the pathological mechanisms triggered by the OPMD mutation we have compared satellite cells from affected and non-affected muscles of the same OPMD patients as well as with cultures from age matched controls, using a proteomic approach. Cytoplasmic and nuclear protein extracts have been separated by 2D gel electrophoresis and differentially expressed proteins have been identified by MALDI-ToF mass spectrometry. We have identified a differential expression of cytoplasmic and nuclear proteins between myoblasts isolated from affected and non-affected muscles, which we are now confirming by WB and immunostaining. The main difference concern proteins involved in signal transduction, intracellular trafficking, energy metabolism, protein folding as well as cell defense. These studies will help us to better understand the pathological processes involved in OPMD to distinguish the mechanisms which are common to normal muscle ageing from those specific of this disease, and to identify deregulated pathways which may represent potential therapeutic targets.

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P2.46
Reduced availability of soluble PABPN1 in a muscle cells is associated with OPMD
S. Maarel, V. Raz
Leiden University Medical Center, Leiden, Netherlands

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disease caused by a GCG repeat expansion mutation in the Poly (A)-binding protein nuclear 1 (expPABPN1). To model the heterozygous situation in OPMD we generated stable mouse myoblasts that express human wild type or mutant PABPN1 at levels similar to endogenous mouse Pabpn1. These low expression levels lead to nuclear aggregation of expPABPN1. For comparison we generated mouse myoblasts stably expressing WT-PABPN1 at similar levels. The molecular relevance of this model to OPMD was demonstrated with a transcriptome comparison between our muscle cell model and skeletal muscles of OPMD patients. Quantitative studies of PABPN1 protein in myotubes revealed that expPABPN1 aggregation and protein accumulation is higher than the WT protein. Differential accumulation of the soluble protein between WT and expPABPN1 was consistent with differences in ubiquitination and protein turnover. We suggest that a lower ratio of soluble to insoluble PABPN1 protein in myotubes expressing expPABPN1 leads to reduced availability of soluble PABPN1 in OPMD muscle weakness.

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DYSFERLIN, ANOCTAMIN 5, AND DISTAL MUSCULAR DYSTROPHIES: POSTER PRESENTATIONS

P2.47
Anoctamin 5 mutations in the Dutch limb girdle muscular dystrophy population
Centre for Human and Clinical Genetics, Leiden University Medical Centre, Leiden, Netherlands; Department of Neurology, Leiden University Medical Centre, Leiden, Netherlands; Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands; Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands; Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands

In The Netherlands a large cohort of 105 patients from 68 families with limb girdle muscular dystrophy (LGMD) has been subject to genetic investigations and in about half of them a causative mutation was pinpointed. Since recently mutations in the Anoctamin 5 gene were identified causing LGMD2L we analysed the remaining LGMD patients for the presence of an ANO5 mutation. Mutation analysis for mutations in the ANO5 gene has been executed in 32 patients. Mutations in the ANO5 gene were found in 7 singleton patients from 7 families. Their age range was 41–61, age of onset varied from 22–39 years, symptoms at onset were related to quadriceps femoris muscle weakness. Serum CK activity was elevated (10–25×). One patient was known with hypertrophic cardiomyopathy, two others had intraventricular septum thickening. One patient suffered from rhabdomyolysis attacks. In The Netherlands, LGMD2L was diagnosed in 10% of all families with LGMD.

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P2.48
Increasing number of new recessive mutations in ANO5: 11 different mutations in 27 patients
S. Penttila, J. Palmio, T. Suominen, B. Udd
University of Tampere and Tampere University Hospital, Neuroumuscular Research Unit, Tampere, Finland

Recently, recessive mutations in ANO5 gene have been shown to be a major cause of limb-girdle and other types of muscular dystrophy. According to recent studies, LGMD2L is one of the most common LGMDs in Europe, its prevalence being similar to the sarcoglycanopathies. The most common mutation in European populations seems to be c.191dupA (p.N64KfsX15). However, in Finland mutation c.2272C > T (p.R758C) has a frequency of 0.35 % and is probably more common. Our sequencing studies of ANO5 have so far revealed 27 muscular dystrophy patients with various mutations. Twenty of these patients were of Finnish origin, three patients were American, two Spanish, one Australian and one Italian. The most common mutation in our patient cohort was c.2272C > T that was homozygous in nine patients and heterozygous in eleven patients. Twelve patients were heterozygous for c.191dupA. In addition we have seen nine other mutations, eight of which are previously unknown. Although most patients had either or both of the common mutations, it is noteworthy that two patients (7%) were compound
Mutations in the anoctamin 5 (ANO5) gene, which encodes a putative calcium-activated chloride channel, have been recently demonstrated to be associated to a new form of limb girdle muscular dystrophy (LGMD), classified as LGMD2L. This is the third most common form of LGMD in the Northern English population and the c.191dupA mutation is responsible for the majority of cases, suggesting a founder effect. From our cohort of LGMD patients (over 203 patients/168 probands) we selected 30 subjects (26 probands), without a molecular diagnosis and studied them trough direct sequencing of all exons and intron boundaries of ANO5. In these patients detailed clinical data were collected and mutations in other LGMD genes were previously excluded. We found mutations in five probands (eight patients). None of them carried the c.191dupA mutation, which is common in Northern and Central Europe countries. Two brothers carried the homozygous duplication c.1627dupA while one subject showed a compound heterozygosity (c. 220C > T and c.1609T > C). In three subjects we found the following heterozygous mutations: c.294G > A, c.1733T > C, c.892G > A. Overall 5 mutations were not previously described. Mutated patients showed mild muscle involvement without cardiac impairment. The majority of them (4/5) showed both proximal and distal weakness and one had a severe respiratory impairment. None lost independent ambulation. Interestingly, three patients showed severe calf-pain-3 deficit at western-blot analysis. Since now only few mutations in ANO5 have been reported and this work enlarges the molecular and clinical spectrum of these disorders. ANO5 gene mutations accounts for about 2.9% of our cohort of Italian LGMDs. Therefore both the occurrence and the molecular epidemiology of ANO5 gene in Italy differ from those observed in other European countries. Further ultrastructural studies will help in understanding pathogenesis.

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