P.149

Differential expression of intermediate filament proteins: lamins A/C and desmin within and between adult skeletal muscles

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The cell cytoskeleton is composed of three main components: microfilaments, microtubules, and intermediate filaments. Intermediate filaments consist of intermediate filament proteins that are widely distributed in cells and tissues, either in the cytoplasm or the nucleus. Desmin is a tissue-specific cytoplasmic intermediate filament protein, while A-type lamins including lamin A/C are ubiquitously expressed nuclear intermediate filament proteins. Both play major roles in skeletal muscle functions. Mutations in the *DES* gene, encoding Desmin, and *LMNA* gene, encoding A-type lamins, lead to a group of rare disorders called desminopathies and laminopathies, respectively. The most frequent pathologies are those involving skeletal muscles. Despite the universal expression of lamins A/C in almost all cells and tissues, different phenotypes associated with laminopathies reflect a high degree of tissue-specificity, the reasons for which are not completely understood. It has also been shown that specific muscles/muscle groups are affected earlier than others in these myopathies. We hypothesized that desmin and lamins A/C are not uniformly expressed in different skeletal muscles and or muscle parts. Accordingly, we have investigated the relative expression of desmin, lamin A & lamin C in 9 skeletal muscles, of which 7 were divided into belly and myotendinous junction (MTJ). Our preliminary results revealed that there is no significant difference in the relative expression of desmin and lamins A/C between bellies and MTJs within a single muscle. We also observed that the relative expression of lamins A & C is correlated with myogenic markers; MyoD and myogenin, while desmin is not. Strikingly, FDB muscle showed the highest expression of lamins A/C compared to other muscles. We are currently investigating desmin, lamin A and lamin C protein levels by western blot and immunohistochemistry and the effect of age and exercise on the expression of these intermediate filament proteins.

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P.150

RNA Sequencing confirms the pathogenicity of a novel *FHL1* deletion

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FHL1 (Four-and-a-half LIM Domains 1) related myopathies are clinically and pathologically heterogeneous. We report a novel pathogenic deletion in *FHL1* gene, whose pathogenicity was clarified by RNA sequencing. An 11-year-old male was referred to our clinic for motor difficulties. At 7 years of age, his parents observed his inability to sit criss cross and unusual kneeling to put on socks/ shoes. A school nurse noted stiff neck. Developmental motor milestones were not delayed. His maternal grandfather had hypertrophic cardiomyopathy. Clinical exam revealed bilateral ptosis, high-arched palate, dysphonia, rigid spine, and thoracic kyphosis. Muscle strength was normal except for 3/5 neck flexion. He had mild flexion contractures of the elbows, knees, and ankles. Serum CK was 256 U/L (upper limit of normal 189 U/L). MRI thighs showed patchy bilateral T2 signal in the gluteus maximus, gluteus medius, and posterior greater than anterior compartment of the thighs with sparing of the bilateral semimembranosus, gracilis, and the right sartorius. Quadriceps muscle biopsy showed mild variation of fiber size. Echocardiogram showed asymmetric septal hypertrophy. A neuromuscular genetic testing panel which included 123 genes revealed a novel hemizygous exon 7 deletion in the *FHL1* gene classified as a variant of uncertain significance. RNA sequencing performed on muscle tissue showed absent junctions to the wild-type exon 7 acceptor splice site, multiple aberrant splicing junctions distal to the location of wild-type exon 7, and significantly reduced expression of *FHL1*. RNA sequencing proved useful in confirming the pathogenicity of a novel *FHL1* deletion. RNA sequencing should be considered in selected myopathy cases to prove or refute the pathogenicity of a detected variant of uncertain significance.

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P.151 - ABSTRACT WITHDRAWN

Myopathy caused by mutations in the *HNRNPA1* gene

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We previously described an adult-onset distal myopathy (MPD3, OMIM #610099) in a large Finnish family with a dominant mode of inheritance. Small hand muscles (intrinsic, thenar and hypothenar) were first involved with spread to the lower legs and later proximal muscles. Dystrophic changes with rimmed vacuoles and cytoplasmic inclusions were observed in muscle biopsies at advanced stage. The causative variant was identified in the *HNRNPA1* gene. This was a heterozygous deletion of the DNA segment chr12:54677979-54678138 spanning the second last exon 10 of the gene. Whole RNA sequencing showed that the main RNA product of the mutant allele results from splicing of exon 9 to 11 and encodes the predicted protein p.Gly356Asnfs*4. Additional likely disease-causing *HNRNPA1* variants were identified in other families with compatible phenotypes. Functional characterization of p.Gly356Asnfs*4 and other novel variants in cell culture models is ongoing to characterize their effects on protein properties and on stress granule dynamics.

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P.152

The novel *ANXA11* variant p.Asp40Ile in a childhood-onset oculopharyngeal muscular dystrophy shows the pathogenic relevance of Asp40 in ANXA11 disorders

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ANXA11 mutations have been associated with two different adult-onset conditions: amyotrophic lateral sclerosis and inclusion body myopathy/multisystem proteinopathy. The pathogenic variant p.Asp40Gly has been identified in individuals with ALS and, more recently, p.Asp40Tyr in patients with multisystem proteinopathy. The pathophysiological mechanism by which the myopathy occurs has not been elucidated until now. Here we describe a patient who carries the new variant of *ANXA11* p.Asp40Ile and shows a severe and rapidly progressive childhood-onset oculopharyngeal muscular dystrophy, mainly characterized by ptosis, ophthalmoplegia, dysphagia, respiratory failure and progressive muscle weakness. The patient's unique early-onset oculopharyngeal muscular dystrophy phenotype is strikingly similar to that recently described in children with frameshift variants of *HNRNPA2B1*, which encodes a ribonucleoprotein involved in dynamic stress granule regulation. Muscle biopsy was characterized by a myopathic pattern that included ANXA11 aggregate and lower levels of both soluble ANXA11 and HNRNPA2B1. Mechanistically, *in vitro* studies using recombinant *ANXA11* p.Asp40Ile revealed defects of the protein function, being the peptide more prone to aggregation. Of note, the impact of p.Asp40Ile was more deleterious on *ANXA11* ability to undergo liquid-liquid phase separation than other mutations affecting the Asp40 residue and associated with other clinical phenotypes. Moreover, ANXA11 and HNRNPA2B1 expression in the patientś fibroblasts was decreased and showed defects in the dynamics of the stress granules. Our findings extend the phenotypic spectrum of ANXA11-related conditions to childhood progressive myopathies and help understand the pleiotropism associated with changes in the aspartic acid in position 40 of *ANXA11*.

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P.153

Pilot trial of sialyllactose in patients with GNE myopathy

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GNE myopathy is a rare inherited muscle disease, characterized by progressive weakness beginning from anterior lower legs and the presence of rimmed vacuoles in muscle pathology. The disease is caused by bi-allelic mutations in *GNE*, which result in reduced sialic acid production. Supplementing sialic acid or related molecules ameliorated the disease progression in the mouse model. In this study, we administered 6'-sialyllactose (6SL) as an alternative source of sialic acid to the patients with GNE myopathy. Twenty patients with GNE myopathy were divided into three groups: 6 into the placebo group, 7 into each 3g and 6g group) for the first 12 weeks. Six from the placebo group switched either to 3g or 6g group for the remaining period. Muscle performance was monitored with the dynamometry and 6-minute walk test. Sialic acid bound to RBC membrane was measured. The patients were requested to fill in the questionnaires every six weeks. Muscle magnetic resonance images (MRI) were taken every 12 weeks to quantify fat content within muscle mass at mid-thigh and mid-calf levels. After 30 weeks, six patients in the initial placebo group showed a significant improvement in muscle power after administration of 6SL for the next 18 weeks, compared with muscle power during the period of placebo medication. Most of the patients in either 3g or 6g group showed no definite aggravation in muscle power, while some of them displayed