P2.21

Dysferlin-mediated membrane repair system contributes to maintenance of skeletal muscle cell viability in mouse models for muscular dystrophy

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Fukuyama-type congenital muscular dystrophy (FCMD) is the second most common childhood muscular dystrophy in Japan. Mutations in the causative gene, *fukutin*, lead to hypoglycosylation and reduced laminin-binding activity of α-dystroglycan. We previously generated the knock-in mice carrying the predominant mutation and showed that a small amount of intact α-dystroglycan is sufficient to prevent disease phenotype. However, a question remained whether any other factors contribute to protect disease manifestation in the fukutin mutant mice. We hypothesized that dysferlin-dependent membrane repair system may contribute to maintain muscle cell viability, and thus generated double-mutant mice defective for both α-DG glycosylation and membrane repair by crossing the fukutin-mutant mice with dysferlin-mutant mice. Histopathological analysis revealed that muscle fibers with centrally-located nuclei, connective tissues, and macrophage infiltration were significantly increased in the double-mutant mice compared to the dysferlin-mutant mice. Dystrophic phenotypes were not detected in the fukutin mutant mice. These data indicate that dysferlin-mediated membrane repair system conceals latent membrane fragility in the skeletal muscles of the fukutin-deficient mice. Furthermore, we show that dysferlin-interacting proteins were up-regulated in Large^{myd} and mdx mice which show progressive muscular dystrophy. These data indicate that the dysferlin-dependent membrane repair complex is activated during progression of muscular dystrophy. Taken together, the present study establishes that membrane repair complex plays an important role to protect or slow disease progression.

doi:10.1016/j.nmd.2011.06.844

P2.22

Quantitative MRI in LGMD2I; a longitudinal study

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Limb Girdle Muscular Dystrophy 2I (LGMD2I) is caused by mutations in the fukutin related protein gene (FKRP). The MRI patterns have been previously been reported. The aim of this study was to (i) assess fat infiltration patterns in the largest, multi-centre cohort of LGMD2I patients using a qualitative radiological score on T1-weighted imaging, (ii) to implement a quantitative 3-point Dixon technique, applied over two time points, developing a non-invasive tool to track the progression of fat infiltration and (iii) to correlate MRI findings with muscle strength and function. The study involves 38 ambulant LGMD2I patients from Newcastle, London, Paris and Copenhagen. The age range is 18–64 years and disease duration, 4–49 years. We have compared the infiltration with matched adult controls. (i) On qualitative T1-weighted imaging the LGMD2I patients showed very few 22 (1.9%) normal (grade 0) muscles and 101 (8.9%) with mild early changes (grade 1), compared to 469 (41.1%) in the top 2 grades of abnormality. Gender changes were demonstrated that have not previously been reported. (ii) Using the 3-point Dixon, quantitative fatty infiltration was calculated and varied significantly from severe (median 70.1%), as in the biceps femoris (long head) to mild (median 5.9%) involvement in the tibialis anterior. The qualitative and quantitative techniques were highly correlated ($r = 0.81$ and $p < 0.0005$). The longitudinal results will be presented. (iii) Strong correlation was seen between the functional and strength tests. In the vastus medialis muscle, a variably infiltrated muscle in both genders, correlations were observed; 6MWD; $r = -0.67$, TUG; $r = -0.61$ and 10 m run/walk; $r = -0.69$. All results were significant at $p < 0.0005$. This study illustrates the usefulness of quantitative MRI in demonstrating pathology, identifying potential target muscles and tracking changes longitudinally. This therefore can be used as a robust, objective and sensitive outcome measure.

doi:10.1016/j.nmd.2011.06.845

LIMB GIRDLE MUSCULAR DYSTROPHIES: POSTER PRESENTATIONS

P2.23

Exploratory Rasch analysis of adapted North Star ambulatory assessment in LGMD 2I

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An adapted North Star Ambulatory Assessment (NSAA) was tested on 16 individuals with LGMD 2I as part of an ongoing natural history study involving MRI (Willis 2011). The group consisted of 12 males and 4 females aged between 8 and 64 (mean age = 37.25 years, median 38 years). The NSAA was adapted from the original designed to measure ambulatory function in Duchenne muscular dystrophy (DMD) which Rasch analysis has shown to be an effective measurement tool. The scale was adapted slightly to suit this population (removed rise from floor item, added squats and Timed Up and Go Test). The test was carried out once on three individuals, twice on two individuals and four times on 11 individuals giving a total of 51 assessments spanning one year. These data were examined using Rasch analysis techniques for Item Fit, Targeting, Clinical cohesiveness, independence, reliability and stability of items to better understand the clinical utility of these items to measure change in this specific group. Results showed important clinical differences in the way the NSAA behaved when compared to its use in DMD. 2/17 items were mildly misfitting and 13/17 items had correctly ordered thresholds. Targeting was reasonable although there was some bunching and gaps on the continuum. In summary, this preliminary analysis shows the adapted NSAA to be a clinically sensible starting point for measuring ambulatory function in LGMD 2I. More data are required to treat these results with more confidence and further studies of its use in other similar NMD would help us improve the scale.

doi:10.1016/j.nmd.2011.06.846

P2.24

Is DNAJB6 part of the chaperone-assisted selective autophagy machinery?

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Recent reports have shown that chaperone-assisted selective autophagy (CASA) is important for correct maintenance of the Z-disk. One of the key components of CASA is HSPA8 (also known as HSC70). HSAP8 is reported to interact with DNAJB6, a co-chaperone of the J-protein family. J-proteins interact with chaperones of the HSPA (Hsp70) family,

modulating and enhancing their activity. DNAJB6 is primarily expressed in non-muscle tissues, but it is present in muscles as well localising to the Z-disks. We have recently identified mutations in DNAJB6 to be the cause of LGMD1D – a disease with marked Z-disk myofibrillar disintegration. We therefore decided to look for potential interactions between DNAJB6 and CASA components by e.g. coimmunoprecipitation of proteins from transfected cell lines and proximity ligation assays on muscle samples. Our results show interaction with most of the complex partners and thus support the association of DNAJB6 with the CASA complex. DNAJB6 is apparently of great importance for correct Z-disk maintenance, possibly via the CASA machinery.

doi:10.1016/j.nmd.2011.06.847

P2.25

Clinical and histological aspects in 17 Brazilian children with sarcoglycanopathy

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Sarcoglycanopathies are a group of autossomal recessive limb girdle muscular dystrophies (LGMD) caused by deficiency in one of the four alfa-, beta-, gama- and delta-sarcoglycans (SG) at muscle fiber sarcolemma. Mutations in individual sarcoglycans are responsible for LGMD-2C (gama-SG), LGMD-2D (alfa-SG), LGMD-2E (beta-SG) and LGMD-2F (delta-SG). The disease is characterized by progressive weakness leading to loss of ambulation, difficulties in breathing and often premature death, associated to high serum creatine kinase (CK) level. Diagnosis of sarcoglycanopathies is based on the histopathological and immunohistochemical analysis of muscle biopsy. Our aim was to report the clinical and histopathological findings in 17 Brazilian children (two pairs of siblings), four males and 13 females, with SG deficiency detected by immunohistochemical analysis. The age at onset of the disease ranged from 2 to 11 years, and the most common clinical presentation were difficulty in climbing stairs, frequent falls and walking on tiptoe. Three patients lost walking ability at 10, 14 and 12 years of age. The weakness predominated in pelvic girdles, and winging of the scapula, calf hypertrophy and scoliosis were also common. CK levels were markedly increased in all patients. Four patients had restrictive respiratory pattern, and three of them presented associated cardiac abnormalities without clinical symptoms. A dystrophic pattern on muscle biopsy was noted in all patients. In 15 of the 17 patients, muscle immunohistochemical analysis was available: all showed reduction or absence of sarcolemmal expression of one, two or more of the four sarcoglycans. Sarcoglycanopathies are severe forms of LGMD affecting predominantly children, and the diagnosis might be confirmed by the detection of the SG deficiency on muscle tissue. Additional molecular testing is required to determine the specific SG deficiency (Sponsored by FAPESP no. 2010/08902-5 and CNPq).

doi:10.1016/j.nmd.2011.06.848

P2.26

LGMD1D mutations impair the antiaggregation activity of DNAJB6

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Mutations in *DNAJB6* have been recently identified as the molecular cause of LGMD1D, a dominant 7q-linked limb-girdle muscular dystrophy with myofibrillar pathology. DNAJB6 is a ubiquitously expressed co-chaperone of the J-domain protein family. The members of this family, in general, function in cellular protein quality control by enhancing and modulating the activity of the Hsp70 (HSPA) chaperones, and some may also have Hsp70-independent functions. DNAJB6, specifically, has been shown to suppress the aggregation of polyglutamine-containing proteins by at least two pathways—one dependent and one independent of the J domain.

To elucidate the functional consequences of the LGMD1D-causing mutations on the antiaggregation activity of DNAJB6, we studied wild-type and mutant DNAJB6 in filter trap assays, using polyglutamine-containing huntingtin as a model client protein. DNAJB6 and huntingtin constructs were coexpressed in T-REx 293 cells, and the ratio of soluble and aggregated huntingtin was determined. Filter trap assays showed that the LGMD1D mutations significantly impaired, but did not completely abolish, the antiaggregation activity of the cytoplasmic DNAJB6b isoform. This loss of activity may at least partially explain the pathogenicity of the mutations.

doi:10.1016/j.nmd.2011.06.849

P2.27

Full exome resequencing by next generation sequencing (NGS) combined with chip analysis for the genetic testing of unclassified myopathic patients

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Exome resequencing by next generation sequencing (NGS) is a very powerful tool to study patients with heterogeneous genetic conditions. The target consists of at least 35 Mb of sequences covering all recognized human exons. At least a 30×–50× coverage is required, especially when searching with possible heterozygous mutations. This strategy has, however, two main problems: (1) each subject shows a huge number of DNA private sequence variations, a part of which (>300 per sample) may be predicted as damaging; (2) copy number variations (CNV) are usually not detected. To answer to these problems we first recruited small familial cases of nonspecific limb-girdle muscular dystrophy or myopathies with apparent autosomal recessive inheritance. At least two affected sibs/family were studied by Illumina 370 K SNP arrays to identify shared segments and crossing over events along the autosomes. In addition, all samples were analyzed by the Agilent MotorChip CGH array. Finally, in 12 cases, composed of a single sample per family, the exome was resequenced using the Solid 3.5 platform. A large number of possible causative mutations were detected, but this number was dramatically reduced by combining SNP data, CNV data and sequence analysis. Finally, we selected a number of 4–20 candidate mutations per sample that were rechecked by Sanger sequencing in all available family members. Using this cost-effective strategy we have identified a number of putative causative mutations. Our results demonstrate that there is a very high genetic heterogeneity in muscular dystrophies and that it is becoming possible to do genetic diagnosis with full comprehensive exome testing.

doi:10.1016/j.nmd.2011.06.850

P2.28

Caveolin-3 and electrical myotonia

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To investigate the molecular basis of a patient with myalgia and electrical myotonia. Caveolin-3 is a major component of the caveolae in skel-