LIMB GIRDLE MUSCULAR DYSTROPHIES II; POSTER PRESENTATIONS

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Exploring the pathogenesis of tibial muscular dystrophy/LGMD2J: Interactions of M-line titin

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Mutations in C-terminal domain M10 of titin, situated in the sarcomeric M-line, cause two muscle diseases. Tibial muscular dystrophy (TMD) is a dominant late onset distal myopathy, caused in Finnish patients by an ins/del mutation (FINmaj) in the M10 domain. In homozygotes, the same mutation causes severe early onset limb-girdle muscular dystrophy type 2J (LGMD2J). Outside the Finnish population, TMD is caused by other missense and nonsense mutations in the same region. The mutations are predicted to cause misfolding or truncation of the Ig-like M10 domain, but epitopes from a larger region are lost from antibody recognition in patient muscle. The molecular pathways leading to TMD/LGMD2J are unknown. The mutations are likely to result in the loss of protein interactions of C-terminal titin, either by directly disrupting the binding or by causing the cleavage of the entire C-terminus. The aim of this study is to determine the protein interactions of M-line titin, possibly involved in the pathogenesis of TMD/LGMD2J. Previously, calpain 3 (CAPN3) was the only known ligand of C-terminal titin. According to our yeast two-hybrid (Y2H) studies, neither the FINmaj nor another missense mutation directly disrupts the interaction, suggesting that cleavage of the titin C-terminus is responsible for the CAPN3 deficiency seen in TMD/LGMD2J. The KY (kyphoscoliosis) protein was also recently found to be a putative ligand of the M10 domain, but our Y2H analyses suggest that the interaction is not physiologically relevant. To identify new ligands of the M10 domain, a Y2H interaction screen was performed. The identified ligand candidates included myospryn (CMYA5), phosphoglucomutase 1 (PGM1), triadin (TRDN), kinectin 1 (KTN1) and ring finger protein 1 (RING1). The putative ligands were analyzed by further two-hybrid studies. While TRDN, KTN1 and RING1 seem unlikely to be true ligands, the interaction of myospryn with M10 has gained additional support. The titin–myospryn interaction seems genuine, as it is disrupted in the Y2H system by the FINmaj mutation. Studies with deletion constructs indicate that the interaction requires a large region of myospryn C-terminus, comprising half of the latter FN3 domain, the SPRY domain and the linker region in between. Protein chemical methods have been used for confirming the interaction. However, the binding has not been conclusively reproduced in vitro, implying that the binding affinity is weak or that protein modifications are needed.

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Cardiac and respiratory involvement in autosomal recessive limb-girdle muscular dystrophies

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Limb-girdle muscular dystrophies (LGMDs) constitute a genetically heterogeneous group of disorders with dominant or recessive inheritance. Their most common presentation is proximal muscle weakness of the pelvic and shoulder girdle muscles and this shapes a variable clinical pattern ranging from severe to very mild muscular involvement. We report data on cardiac and respiratory involvement in 89 out of 180 patients with severe forms of autosomal recessive LGMDs followed at the Cardiomyology and Medical Genetics of Second Naples University, sharing mutations in CAPN3 (49), alpha, beta, gamma and delta-sarcoglycans (25), FKRP (9) and DYSF (6) genes, evaluated by clinical, electro-cardiographic, echocardiographic, scintigraphic and spirometric assessments. No cardiac involvement was found in patients carrying mutations in CAPN3, FKRP and DYSF genes. Conversely in sarcoglycan mutated patients, 31.1% showed a normal heart, 43.7% preclinical cardiomyopathy, 6.3% arrhythmogenic cardiomyopathy and 18.7% signs of dilated cardiomyopathy. With reference to the type of sarcoglycanopathy, dilated cardiomyopathy occurred in gamma and delta-sarcoglycanopathies, in which the evolution of cardiomyopathy was the same we observed in dystrophinopathic patients. No correlation was found between cardiac and skeletal muscle involvement. A normal respiratory function was observed in 19.1% of all patients, a mild impairment (Vital Capacity between 85 and 65%) in 43.9%, a moderate impairment (Vital Capacity between 65 and 40%) in 23.5%, and a severe impairment (Vital Capacity $< 40\%$) in 13.5%. With reference to the type of LGMDs, the more severe pictures of restrictive dysventilatory syndrome occurred in FKRP mutated patients (about 2/3 of cases). Patients affected by dysferlinopathy seem to be spared.

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Incidence of 550delA in the CAPN3 gene in German patients with limb-girdle muscular dystrophy and hyperCKemia

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Calpain-3 deficiency is the most common cause of autosomal-recessive limb-girdle muscular dystrophy (LGMD2). The 550delA mutation in the CAPN3 gene was frequently identified in patients from Eastern Europe. A screening for the 550delA mutation was performed in unrelated German patients with LGMD2 ($n = 98$) and unknown hyperCKemia ($n = 106$). Western blot analysis was available in 76 patients with LGMD2 and 35 patients with hyperCKemia. In samples that were heterozygous for the 550delA mutation the whole coding region of the CAPN3 was analyzed by sequencing. 550delA was found in 8.1% in LGMD2 ($n = 1$ homozygous, $n = 7$ heterozygous) and 1.9% in hyperCkemia ($n = 2$ heterozygous). In 8/9 heterozygous patients another CAPN3 mutation on the second allele was identified. Two mutations (Val509Phe and Gln565Stop) are novel. Pathological calpain-3 protein was found in 30% of the LGMD2 patients and 17% of the patients with hyperCKemia. Analysis of Calapin-3 protein by Western blotting was available in 9 out of the 10 patients with genetically confirmed LGMD2A and was clearly abnormal in 6 patients, suspicious in two and totally normal in one. Two patients with 550delA mutation and onset within the first two decades had joint contractures. The CAPN3 gene 550delA mutation is frequently identified in German patients with LGMD2, but also in rare cases with hyperCkemia. Thus, investigation of the 550delA mutation can be a helpful screening method. However, our observations indicate that highest sensitivity in diagnosis of