homogeneous clinical presentation. In our cohort, the clinical phenotype was heterogeneous and the severity of symptoms varied extensively between patients even with the same mutation. The only common clue to diagnosis was a clearly high CK level.

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P2.52

Dysferlin and anoctamin 5 mutations in the Dutch distal muscular dystrophy cohort

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In 1997 we published the results of a cross-sectional study on 24 Dutch phenotypic Miyoshi myopathy (MMD) patients. We showed that 30% of the patients became wheelchair-bound within 10 years after onset of the disease. At that time the genetic background of the patients was unknown. Analysis for mutations in the dysferlin and the anoctamin 5 gene (ANO5) has been performed in 23 patients. In addition, the patients were followed up. Mutations in the dysferlin gene were found in 10 patients from 8 families (MMD1). Previously, in 4 patients from 2 families linkage with chromosome 10 was found (MMD2). Mutations in the ANO5 gene were identified in 7 patients from 5 families (MMD3). In the remaining 2 patients with a Miyoshi-like clinical picture as yet no mutation was found. Age of onset in the MMD1 group varied from 18–51 years, and in the MMD3 group from 17–40 years. Five out of 10 patients with MMD1 were wheelchair bound as opposed to the MMD3 patients who were all still ambulatory. Mutations in dysferlin seem to give rise to a more severe distal muscular dystrophy phenotype as compared to mutations in anoctamin 5.

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P2.53

The mdx/SJL mouse: A new double mutant model for neuromuscular disorders with mutations in the dystrophin and dysferlin genes

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Muscular dystrophies (MD) are genetic disorders, still devoid of treatment. The Dmd^{mdx} is the classical model for Duchenne MD. A point mutation in the dystrophin gene results in the total absence of the protein in his muscle. Interestingly, regeneration is highly activated in this model, resulting in a very mild phenotype. The SJL/J mouse is a model for LGMD-2B, with a deficiency of dysferlin, a protein associated with membrane repair. Although also mildly affected, this model shows signs of weakness in forced swimming and tail suspension functional tests. Cell therapy trials have been successfully done in this model. To increase our knowledge on the role of both proteins in the degeneration and regeneration processes, as well as to create mouse models with appropriate characteristics for testing putative therapies, we generated by Mendelian crosses double mutants for both dystrophin and dysferlin proteins. Among 54 litters more than 140 double affected animals were obtained. Genotype was confirmed through DNA and protein analyses. Neither intrauterine death nor reproductive abnormalities were observed, and we are at this point in the F6 generation. Up to now, functional evaluation is showing no significant weakness. Muscle histopathological findings include the prevalence of centronucleated fibers. More studies are ongoing to verify the effect of deficiency of both proteins in muscle membrane repair, under an active regeneration process. Additionally, this new mouse model will be used in experiments of cell therapy, since the retention and differentiation of injected cells can be tracked through the presence of exogenous dystrophin. We are also turning this strain isogenic with more cross breeds. Longitudinal study of their functional ability will elucidate the effect of the Dmd^{mdx} mutation in a different genetic background. FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.

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P2.54

Real time analysis of sarcolemmal repair mediated by dysferlin and MG53

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Dysferlin is a sarcolemmal protein that is defective in MM and LGMD 2B. In the presence of Ca2+, dysferlin accumulates around the injured site and is suggested to mediate sarcolemmal repair. Mitsugumin 53 (MG53) is also involved in membrane repair in skeletal muscle and recruited around wounded sites in an oxidation-dependent manner. Recently, it was reported that MG53 interacts with dysferlin and caveolin-3 and regulates membrane repair in skeletal muscle. To clarify molecular behavior of dysferlin and MG53 during sarcolemmal repair. Immunoprecipitation was performed using COS-7 cells transfected with WT or mutant (W999C, V67D and W52R) dysferlin-c-myc and FLAG-MG53. The p.W999C mutation is located in DysfN domain, and the p.W52R and p.V67D mutations are located in C2A domain of dysferlin and are reported in MM and LGMD2B patients. We expressed dysferlin (WT, V67D and W52R)-EGFP and/or MG53-mCherry in skeletal muscle myocytes for whole-mount viewing or individual myofibers isolated from it, and then subjected them to a plasma membrane disruption created by a 2-photon laser. IP study revealed that WT and W999C dysferlin associates with both MG53- monomer and oligomer whereas both C2A-mutant dysferlins alter association with MG53-monomer and oligomer. Confocal imaging revealed a striking and rapid (second time-scale) accumulation of dysferlin-EGFP at the disruption site, followed by accumulation of MG53-mCherry (second time-scale). WT and both C2A-mutant dysferlins accumulate around injury site. We also observed, for the first time in living muscle myocytes responding to a membrane disruption, monomerization and/or oligomerization of MG53 events at the disruption site using redox-sensitive GFPs. We hypothesize that rapid change of MG53 formation followed by accumulated dysferlin in the way of the membrane-membrane contacts leading to the homotypic and exocytotic fusion events required for repair.

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P2.55

Mstn/Dysf double knockout mice gain muscle mass but no strength

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Mutations in myostatin lead to a massive increase in muscle mass suggesting potential relevance of myostatin inhibition for therapeutic treatment of muscle wasting. Contradictory data in mdx and laminin a2 deficient mice have been published debating on the effect of myostatin inhibition in case of muscular dystrophies. Mutations in dysferlin cause limb girdle muscular dystrophy 2B due to defects in muscle membrane repair. We asked whether myostatin knock-out in a dysferlin deficient mouse model leads to improved muscle performance and a decrease in histopathological alterations. We crossed myostatin null mutant mice with the dysferlin deficient mouse model B6.A/J-Dysf^{prmd}. Mstn $^{-/-}$/ B6.A/J-Dysf^{prmd} (DKO) were compared to Mstn$^{-/-}$, B6.A/J-Dysf^{prmd} and C57BL/6 mice ($n = 8$/group). Individual muscle mass was determined and body composition was performed. Treadmill performance was assessed for three weeks and grip strength was tested. We histologically analyzed Quadriceps and Tibialis muscle of untrained and trained mice at three months of age. Mstn$^{-/-}$ and DKO mice equally gained muscle mass but differed significantly in physiological muscle function. In treadmill analysis B6.A/J-Dysf^{prmd} slowly worsened over time (weeks) compared to WT and Mstn$^{-/-}$ while DKO already showed a significantly reduced running distance and a higher rate of drop backs during the first training days. Grip strength was mostly reduced in DKO, but was also significantly diminished in B6.A/J-Dysf^{prmd} and Mstn$^{-/-}$ compared to WT. The histological analysis reflects the impaired muscle function of B6.A/J-Dysf^{prmd} and especially DKO mice with significantly increased numbers of central nucleated, necrotic and regenerating fibers. Like in Mstn$^{-/-}$ mice muscle fiber diameter was increased in DKO mice but showing additional atrophic fibers. These results indicate that permanent myostatin knock-out aggravates the course of disease in dysferlinopathy and that this is not a promising therapeutic option.

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P2.56

Migration of an ancestral dysferlin splicing mutation from the Iberian peninsula to South America

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Miyoshi myopathy, LGMD2B and DMAT are primary dysferlinopathies that belong to a group of muscular dystrophies inherited in an autosomal recessive mode. Additional presentations range from isolated hyperCKemia to severe functional disability. LGMD2B involves predominantly the proximal muscles of the lower limbs whereas in Miyoshi myopathy the muscles involved are those of the posterior muscle compartment of the calf. DMAT is characterized by anterior tibial muscle weakness which rapidly progresses to the lower and upper proximal muscles. Onset is usually in young adults, but congenital and late-onset forms have also been reported. We present the first Uruguayan patient to have been diagnosed with Miyoshi myopathy and four Portuguese patients that carry a novel mutation in exon12/intron12 boundary: c.1180_1180 + 7delAGTGCGTG (r.1054_1284del) in the DYSF gene. Evidence of a founder effect due to a common ancestral origin of this mutation was detected in heterozygosity in four patients and in homozygosity in one patient. The homozygous patient has no proven inbreeding so it can be inferred that the mutation is identical by descent. All patients shared a common haplotype block identical in state between markers Cy172-H32 and D2S211. We believe that it derives from a common mutational event which is ancestral because of the recombination between the mutated gene and the telomeric flanking marker D2S2113. As this haplotype is not common among the Portuguese population, it is very unlikely that these mutated DYSF alleles represent recurrent events. This is the sixth founder effect of the DYSF gene to be found in the world so far.

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P2.57

First dysferlinopathy patients in Egypt: Clinical, pathological and genetic characteristics

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Dysferlinopathy is caused by mutations of the dysferlin gene (DYSF) on chromosome 2p13. To study the clinical, pathological and genetic characteristics of dysferlinopathy in Egypt. Patients were selected from those with progressive muscular dystrophy referred to Muscle and Nerve Research Laboratory, Cairo, Egypt. We studied 77 patients with dystrophic muscle biopsy. Patients had neurological assessment, family pedigree study, Serum Creatine Kinase, ECHO cardiography, electromyography and Dystrophin gene testing. A battery of histochemical tests, immunohistochemistry using both fluorescent and automated methods against a panel of antibodies were done. Mini-multiplex Western Blotting was also done. Genetic study of Dysferlin gene was done for some patients. We found 40 patients with limb-girdle muscular dystrophy, 12 with dysferlinopathy, 6 with calpainopathy, 6 with sarcoglycanopathy and 16 with non-specified limb-girdle muscular dystrophy. Dysferlinopathy patients showed 3 patients with proximal type, 3 patients with Miyoshi myopathy and 6 patients with anterior compartment myopathy. Weakness was asymmetrical in 5 patients and symmetrical in 7 patients. Some muscles were uniformly affected in most patients: gluteus maximus, hamstrings, gastrocnemius, tibialis anterior and deltoids. Pathological study showed dystrophic changes of all biopsies, mitochondrial changes in 2 biopsies and inflammatory changes in 3 biopsies. Dysferlin gene analysis was done for only 6 patients of 4 families, showed homozygous mutations in two families, one heterozygous mutation in one family, and no mutation (only polymorphisms) in one family. Dysferlinopathy is a common condition in Egypt. Many clinical, pathological and genetic characteristics are found and help in its diagnosis.

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P2.58

Dissecting the interactions of proteins constituting the dysferlin complex

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Dysferlinopathies are a group of progressive muscular dystrophies characterized by mutations in the gene DYSF causing a severe reduction or complete absence of the protein dysferlin. It is expressed mainly in skel-