involved disclosed no abnormalities. The child suffered transient torticollis-like position at 10 months of age. On evaluation at the age of 16 months, she was an active girl, with proximal weakness without muscle atrophy. CK level elevated (1037 U/L); EMG showed myopathic features. Muscle biopsy was indicative of a congenital muscular dystrophy with prominent subsarcolemal inclusions and abnormal nuclei. Dystrophin, sarcoglycans, z2-laminin, z- and β-dystroglycans, emerin, lamin A/C, nesprin1 and SUN2 expression was well preserved. However, the expression of SUN1 a protein of the nuclear envelope was absent in some nuclei. Under the electron microscopy the most striking finding was the presence of prominent abnormal nuclei containing hypercondensed chromatin and abundant invaginations of neighboring cytoplasm. Several nuclei appeared completely disintegrated and replaced by large collections of electrondense granular material. Sequence analysis of coding regions of LMNA gene revealed no mutations; SUN1 gene is currently being screened. We describe a new form of early onset myopathy with striking so far unreported nuclear abnormalities widening the group of myopathies with abnormal nuclei, therefore disorders of muscle nuclei should be considered in patients with unknown early onset muscle disorders. Further investigations are required to understand the pathogenic events leading to this peculiar myopathy.

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P2.11

A new form of myopathy associated with muscle hypertrophy, short stature, macroGLOSSia and brachydactyly

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An 11 years-old girl was seen in our Center presenting short stature, learning disabilities, muscle weakness and global and significant muscle hypertrophy. The mother referred that her first daughter died at 9 months of age, with a similar phenotype, suggesting an autosomal recessive inheritance. Complementary exams disclosed normal levels of triglycerides, cholesterol, renal function, calcium, phosphorus, 25-vitamin D, GH, IGFI, IGFBP3, T4, TSH, PTH. Skeletal survey showed partial C5-C6 vertebral fusion, mild scoliosis and shortness of the 3rd, 4th and 5th metacarpals, and the 1st metatarsal. Abdominal and pelvic ultrasound, as well as ophthalmologic evaluation, including fundoscopy and slit-lamp exams were normal. Neurological evaluation showed a generalized and marked muscular hypertrophy, proximal weakness in limbs and presence of Gowers sign. Osteo tendineous reflexes were abolished. The coordination was normal. There was neither grip nor percussion myotonia. Karyotype and CGH-array were normal. Muscle biopsy showed an active dystrophic process, and protein analyses revealed a normal pattern for dystrophin, dysferlin, calpain3, the 4 sarcoglycans, teletomin and z2-laminin. A detailed ENMG showed a pattern compatible with myopathy, with no signs of myotonia. Therefore, all forms of congenital myotonias were excluded. Based on her hypermusculature, a possible alteration in myostatin expression was suspected. However, no mutations, nor polymorphisms were observed by sequencing the myostatin gene. Western blot analysis detected the presence of the precursor and mature peptides bands of myostatin. Alterations in other proteins involved in myostatin function could not be excluded yet. A clinical follow up and additional studies are ongoing to elucidate the diagnosis of this atypical case. FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.

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P2.12

Generation of a new mouse model for therapeutic testing in the dystroglycanopathies

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Mutations in fukutin related protein (FKRP) are responsible for a common group of muscular dystrophies ranging from adult onset limb girdle muscular dystrophies to severe congenital forms with associated structured brain involvement, including Muscle Eye Brain Disease. We have previously generated a mouse with a knock-down in Fkrp expression levels (FKRPKD) due to insertion of a floxed neomycin cassette in intron 2 of the mouse Fkrp gene. Since this mouse dies at birth due to central nervous involvement we have now replaced FKRP activity in the developing neural tube by crossing this line with one expressing Cre recombinae under the Sox-1 promoter. This has resulted in a viable mouse model in which glycosylated z-dystroglycan and laminin alpha 2 has been restored at the pial basement membrane in the brain. These mice display a marked reduction in the glycosylation of skeletal muscle z-dystroglycan (ADG) throughout postnatal life and develop a clear muscle phenotype (fibre degeneration and regeneration) in both the limb muscles and the diaphragm by 12 weeks of age. This new model should prove invaluable in the design and testing of future therapeutic strategies in the dystroglycanopathies.

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P2.13

Further characterization of the clinical and mutational spectrum of alpha-dystroglycanopathy caused by mutations in the LARGE gene

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Alpha-dystroglycanopathies (aDGPath) constitute a subset of the congenital muscular dystrophies and encompass a spectrum of clinical severity, from Walker-Warburg syndrome (WWS) to a milder, LGMD-like muscular dystrophy. Seven genes have been implicated in aDGPath: POMT1, POMT2, POMGNT1, FKTN, FKRP, LARGE, and DAG1. Six patients in 4 families have been reported with mutations in LARGE. We recently identified two additional unrelated aDGPath patients with LARGE mutations, expanding the disease spectrum. Clinical examination, brain MRIs, and genetic testing on two patients presenting with an aDGPath phenotype. The first patient also underwent a muscle biopsy. Patient I: 5 year old female with esotropia, mild proximal weakness, able to hop and run, significant cognitive and speech delays. Normal eye examination. Muscle biopsy: mildly dystrophic, severe reduction of glycosylated alpha-dystroglycan on immunostaining and Western blot. CK: 2230 IU/L. Brain MRI: Regionally pachygyric cortex, patchy white matter abnormalities in high parietal white matter, slightly small pons, inferior vermis hypoplasia, no cerebellar cysts. Genetic testing: Novel 76 kb deletion containing part of LARGE gene and previously reported missense mutation on the other allele p.Glu509Lvs. (c.1525G > A). Patient II: 5 month old female born to consanguineous parents, dysmorphic face, dysconjugate
gaze, head lag, facial and truncal hypotonia. Respiratory insufficiency, reflux. CK: 3099 IU/L. Brain MRI: lissencephaly type 2, hydrocephalus, absent septum pellucidum, consistent with WWS. Genetic testing: novel homozygous variant, c.1328_1329delGInsAT (p.Cys443Tyr). We identified two additional patients with aDGopathy due to mutations in LARGE, providing additional characterization of the clinical and genetic spectrum of aDGopathy. Given the severity of the second patient, the Cys443 residue may be functionally important, yet its exact function remains to be clarified.

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P2.14
The versatility of flow cytometry in the assessment of functional alpha-dystroglycan glycosylation
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Alpha-dystroglycan (ADG) is a peripheral membrane protein that is an integral component of the dystrophin glycoprotein complex. It is a link between the intracellular cytoskeleton and extracellular matrix molecules such as laminin. In an inherited subset of muscular dystrophies known as the dystroglycanopathies, ADG has reduced levels of glycosylation which results in lower laminin binding, as detected by the anti-dystroglycan antibody IIH6 and blot overlay studies. The IIH6 antibody can serve as a biomarker for these disorders as it binds a specific glycan epitope on ADG involved in the laminin binding. In this study flow cytometry was used to assess the level of IIH6-reactive glycans in myoblasts, for the identification of compounds capable of increasing the glycosylation on ADG and therefore to restore binding with its physiological partners. We used myoblasts derived from the H-2Kb-ts A58 transgenic mouse to screen an iminosugar library to identify hits capable of inducing ADG hyperglycosylation. Iminosugars are synthetically tractable hexose mimics capable of modulating carbohydrate processing. Additionally, flow cytometry experiments were conducted to compare the level of functional glycosylation in fibroblasts from control and dystroglycanopathy patients. This was done to see if the amount of IIH6 positive glycans could be accurately determined and quantified by this method both to aid diagnosis and as a baseline for future glycosylation restoration studies. Our results show that myoblasts glycosylation status can be readily and reproducibly identified using flow cytometry. Furthermore control fibroblasts have clearly detectable levels of glycosylated dystroglycan and we are currently assessing patients’ cells to assess if this test could complement existing diagnostic assays. The screening of the iminosugar library could allow for the identification of novel chemical starting points which may lead to new therapies for this group of diseases.

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P2.15
Assessing the therapeutic potential of LARGE in a new mouse model of dystroglycanopathy
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Mutations in fukutin related protein (FKRP) are responsible for a common group of muscular dystrophies ranging from adult onset limb girdle muscular dystrophies to severe congenital forms. We have now generated a mouse with a knock-down in Fkrrp expression levels in the skeletal muscle but not the central nervous system (Sox1 Cre FKRPKD). The skeletal muscle of this mouse shows a marked reduction in glycosylated dystroglycan and develops a clear muscle phenotype by 12 weeks of age. Previous work has shown that the over-expression of LARGE induces the hyperglycosylation of α-dystroglycan in both wild type and in cells from dystroglycanopathy patients, irrespective of their primary gene defect, strongly suggesting that LARGE could be an important therapeutic approach in these disorders. As a first step to confirming this on a disease background in vivo, we have now crossed the Sox1Cre FKRPKD line with one over-expressing LARGE. We present here our histological and physiological evaluation of the resulting phenotypes.

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P2.16
Deposition of the inner limiting membrane in the eye of a mouse model for Muscle Eye Brain disease
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Dystroglycan is composed of two subunits; β-dystroglycan (transmembrane) and α-dystroglycan (peripheral membrane protein), the latter of which binds to several extracellular matrix proteins including laminin, perlecan and agrin. α-Dystroglycan is extensively glycosylated and mutations in at least 8 known genes have so far been shown to be associated with defective dystroglycan processing and a phenotype now commonly referred to as a dystroglycanopathy. Mutations in one of these genes, fukutin related protein or FKRP is associated with a wide clinical spectrum, the most severe of which includes Walker Warburg Syndrome and Muscle Eye Brain disease both of which are associated with eye abnormalities. To further investigate the underlying basis for this we used a FKRP knock-down mouse model to examine the pattern of α-dystroglycan glycosylation and associated basement membrane proteins at the inner limiting membrane (ILM) from E12.5 up until birth (P0). We demonstrate that the glycosylation of α-dystroglycan is developmentally regulated and that glycosylation defects in the FKRP knock-down mouse is associated with breaches in the ILM during development.

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P2.17
Fukutin-related protein resides in the Golgi cisternae of human skeletal muscle fibres and forms disulfide-linked homodimers via an N-terminal interaction interface
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Limb-Girdle Muscular Dystrophy type 2I (LGMD2I) is an inheritable autosomal, recessive disorder caused by mutations in the FucKuin-Related Protein (FKRP) gene (FKRP) located on chromosome 19 (19q13.3). Mutations in FKRP are also associated with congenital muscular dystrophy (MDC1C), Walker-Warburg syndrome (WW) and Muscle Eye Brain disease (MEB). These four disorders share in common an incomplete/