G.P.3
Modeling congenital muscle diseases in zebrafish
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Primary inherited disorders of muscle include both dystrophies and non-dystrophic congenital myopathies. These disorders, characterized by muscle weakness and impaired locomotion, form a heterogeneous group of hereditary diseases affecting both children and adults. Their complexity is highlighted by occasional involvement of associated symptoms in brain, eyes, heart or respiratory system. Owing to disease heterogeneity, variable penetrance, and mortality, genetic studies to determine the basis for these conditions in humans are often problematic. The zebrafish, Danio rerio, is a powerful developmental and genetic model system for dissection of skeletal muscle disorders and diseases. Several features of zebrafish, such as high degrees of synteny and sequence homology, conserved structure of skeletal muscles, transparency at embryonic stages, and rapid ex vivo development, make them ideal for skeletal muscle biology research. To identify novel genes causing muscular disorders, we conducted an ENU mutagenesis screen in zebrafish that led to the identification of 12 unique neuromuscular mutants. One of these represents a model of the dystroglycanopathies (dagl) and another, of congenital muscular dystrophy (l ama2). Another mutant identified in this screen, oosil, displays impaired motility behavior and skeletal muscle myotonia. These mutant fish show skeletal muscle hypertrophy with extensive central neculation. Ultrastructural studies revealed sarcomeric disorganization in ososi fish. Genetic mapping of the oosi locus has identified a loss of function mutation in a novel RNA binding protein implicated in RNA splicing. Preliminary studies point towards a role for this gene in the disease pathogenesis of myotonic dystrophy. Through an understanding of the abnormal skeletal muscle molecular pathways in ososi fish, we can better understand human neuromuscular diseases and begin to develop corrective therapies.

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G.P.4
A comparison of the structural brain defects in dystroglycanopathy mouse models
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Mutations in several genes encoding either a determined or putative glycosyltransferases are associated with the hypoglycosylation of α-dystroglycan, which effectively disrupts the linkage between the dystrophin-associated glycoprotein complex and the extracellular matrix. These mutations are responsible for a clinically heterogeneous group of muscular dystrophies, which vary in severity from severe congenital muscular dystrophies, with substantial brain and eye involvement, to relatively mild adult-onset limb girdle muscular dystrophies. The underlying reason for the spectrum of defects, ranging from complete lissencephaly in patients with Walker–Warburg syndrome to isolated cerebellar involvement is at present unclear. In order to further investigate this aspect we have undertaken a comparative study of structural brain involvement in different dystroglycanopathy mouse models, with a particular emphasis on the deposition of the pial basement membrane. Our data indicate that several processes contribute to the disease process and that there are important differences between models.

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G.P.5
Over expression of the LARGE transgene exacerbates muscle pathology in the mdx mouse
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Duchenne muscle dystrophy is an X-linked muscle wasting disorder caused by mutations in the DMD gene preventing the production of a functional dystrophin protein. The lack of dystrophin de-stabilises the dystrophin associated protein complex (DAPC) and consequently DMD muscles are more susceptible to exercised induced muscle damage. We hypothesised stabilising the DAPC at the sarcolemma in mdx mice may reduce muscle susceptibility to exercise induced damage and consequently ameliorate muscle pathology. To evaluate this we crossed mice over-expressing the putative glycosyltransferase, LARGE, which facilitates the binding of alpha dystroglycan to laminin in the extracellular matrix, with mdx mice. The presence of the LARGE transgene in the LVS/mdx mice was established through genotyping and expression was confirmed through immunostaining. Pathological findings characterised by myofibre size variation, increased central neculation, presence of inflammatory cells and calcium deposits was more serve in 8-week old LVS/mdx mice quadriceps and diaphragm muscles compared to mdtermate controls. To determine if the over-expression of the LARGE transgene initiated muscle pathology early we analysed 3-week old quadriceps muscles. Stark degeneration was observed in LVS/mdx vastus muscles, in contrast to the rectus femoris which was well preserved. Few pathological findings were observed in the age matched mdx littermate controls. We also assessed the effect of over-expression of LARGE on muscle function using a standardised exercise test on the tibialis anterior muscle in situ. A significant drop in maximum force produced following eccentric contractions was observed in 22-week old LV5/mdx mice compared to littermate controls. Overall we show the over-expression of LARGE exacerbates DMD pathology in the mdx mice. Further work to establish an underlining mechanism for the deleterious effect of over-expressing LARGE in mdx mice is currently ongoing.

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G.P.6
Assessing the long term expression of LARGE as a potential therapy in a mouse model of LGMD2I
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Mutations in at least eight genes, including 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 structural brain involvement including Walker–Warburg syndrome. We have now generated a mouse with a knock-down in Fkkr expression in the skeletal muscle, but not the central nervous system (FKRPM). Analysis of skeletal muscle form this mouse demonstrates hypoglycosylation of α-dystroglycan and a clear muscle pathology by 12 weeks of age. Previous work has shown that LARGE overexpression induces hyperglycosylation of α-dystroglycan in wildtype cells and additionally cells from dystroglycanopathy patients with various primary gene defects, suggesting LARGE could be an important therapeutic approach in these disorders. To test this strategy for FKRRP associated disorders, we have crossed our FKRPM MD mouse line with a second line overexpressing LARGE. Here we present our
histological and physiological evaluation of these mice (FKRPMDLARGE).

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G.P.7
Effects of overexpression of LARGE on a mouse model of congenital muscular dystrophy
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Dystroglycan (DG) is a central component of dystrophin–glycoprotein complex that links extracellular matrix and cytoskeleton. α-Dystroglycanopathy is a disorder characterized by muscular dystrophy often associated with brain anomaly and eye abnormalities. Mutations of known or putative glycosyltransferases, POMGnT1, POMT1, POMT2, fukutin, FKRP, LARGE, and LARGEN, were identified and defective glycosylation of α-DG was implicated in the pathogenesis of this disorder. Interestingly, it has been reported that overexpression of LARGE strongly increase a function of α-DG and bypasses the defective glycosylation of α-DG in cultured cells deficient in fukutin or POMGnT1. Most recently, it has been demonstrated that LARGE acts on α-DG as a bifunctional glycosyltransferase which produces repeating units of xylose and glucuronic acid. In the previous study, we have generated transgenic mice that overexpresses LARGE (LARGE Tg mice) and characterized them. LARGE Tg mice were born according to the Mendelian ratio and grew normally. The mice exhibited no obvious abnormal behavior. In the mice, α-DG was hyperglycosylated and its laminin binding activity was greatly increased ubiquitously. Because the enhanced binding of α-DG with laminin should increase the stability of sarcolema and facilitate signal transduction through α-DG, it is conceivable that the overexpression of LARGE could ameliorate the degeneration of muscle cells even in other types of muscular dystrophy than α-dystroglycanopathy. In this study, we crossed LARGE Tg mice with dy2J mice, one of a model mouse of congenital muscular dystrophy 1A in which laminin α2 chain is deficient. The dy2J mice over-expressing LARGE (dy2J/LARGE mice) have been born and we are currently characterizing them. In this meeting, we will present the detailed phenotype of dy2J/LARGE mice particularly focusing on its muscle pathology.

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G.P.8
Expression analysis of α-dystroglycan glycosyltransferases in distinct murine muscular dystrophy models
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Dystroglycanopathies are genetic muscular dystrophies caused by defects in the glycosylation of α-dystroglycan (DG), an important component of the dystrophin-associated glycoprotein complex (DAG). There are at least six proven or putative glycosyltransferases enzymes, related to muscle diseases, which play a role in O-mannosyl-linked glycosylation of α-DG. In order to address the roles of α-dystroglycan glycosylation in distinct pathways of muscle degeneration and during muscle development we investigated the relative expression of four of them: POMT1, POMGnT1, LARGE, and FKRP, in the gastrocnemius muscle from four mouse models for muscular dystrophies: Dmdmdx, Lama202/21J, Largeemd, SJL/J, as compared to normal C57Black6 lineage. The study was done through real time PCR quantification, in three different ages: new born, 3 and 6 months of age. In normal mice, a decreased expression with the age was observed for all genes, mainly for Fkrr and Large. In dystrophic lineages, we observed a large variation in the expression of the four glycosylation genes, as compared to normal age-matched mice. In new born, these differences were more significant and an upregulation was observed in Dmdmdx and Largeemd strains, while a downregulation, was detected in Lama202/21J and SJL/J strains. In adult animals, the pattern was more close to normal mice of the same age. Our results suggest that an increase in α-dystroglycan glycosylation occurs during muscle development, and possibly also during the process of muscle regeneration. However, this process is variable in the diverse dystrophic strains.

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G.P.9
Basement membrane pathology associated with FKRP and fukutin deficiency in zebradish
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Deficiencies in fukutin-related protein (FKRP) and fukutin lead to aberrant glycosylation of alpha-dystroglycan, a key receptor for basement membrane proteins. There is a broad spectrum of disorders associated with FKRP and fukutin deficiency, ranging from limb-girdle muscular dystrophy to the congenital muscular dystrophy syndromes Muscle Eye Brain disease and Walker–Warburg syndrome. Here we use zebradish to investigate the changes in several basement membranes in muscle, eye and notochord using transmission electron microscopy and immunohistochemistry in 3 days post fertilisation larvae. A range of antibodies including, IIH6, laminin, zn-8 (optic nerve), zraf-1 (Muller cells) were used to study the notochord and eye phenotypes on cryosections. FKRP and fukutin were knocked down by antisense oligonucleotide morpholinos in wild type zebradish. The morphants showed abnormal muscle fibres and disrupted vertical myosepta. Disturbances were observed in all three layers that form the peri-notochord sheath including the basement membrane. Disorganised retinal layering in both morphants was seen on toluidine blue stained sections. Dysplasia of the lens could be observed in both fukutin and FKRP morphants with a severe phenotype. The homogenous perturbation observed across the inner limiting membranes of both morphants may account for the lens dysplasia. The rod and cones in the photoreceptor cell layer were found in lower density in both morphants with the least density in fukutin knock-downs which may be the result of a disrupted external limiting membrane. We therefore conclude FKRP and fukutin are essential for the integrity of membranes in the eye, muscle and notochord of developing zebradish larvae.

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G.P.10
Basement membrane deposition in the skeletal muscle of the FKRP knock-down mouse
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