NMJ on both LARGE overexpressing mice and FKRPKD were significantly higher than the controls, without changing the total volume or the surface area of the junction. Colocalization of the presynaptic marker synaptophysin and AChR clusters of the postsynaptic apparatus was generally maintained in all groups, and only lost in some NMJs of the FKRPKD mice. We studied potential denervation induced processes by NCAM staining and by labelling axons emerging from the endplates with neurofilament antibodies. Finally, we characterize the expression of agrin around the NMJs as one of the glycoproteins that has been shown to be a key player on the aggregation and clustering of the AChR receptors at the NMJs.

http://dx.doi:10.1016/j.nmd.2013.06.508

P.8.8
Basement membrane deposition during muscle development in the FKRP Deficient Mouse
J. Kim, M. Fuente-Fernandez, M. Kavishwar, S.C. Brown
Royal Veterinary College, Comparative Biomedical Sciences, London, United Kingdom

The defective glycosylation of α-dystroglycan is associated with a group of muscular dystrophies collectively referred to as the dystroglycanopathies. We previously generated a FKRP deficient mouse (FKRPKD) which recapitulates the severe end of the clinical spectrum and displays a muscle/eye/brain phenotype and dies soon after birth. In view of the well documented role of α-dystroglycan in basement membrane deposition we sought to determine if secondary myogenesis was altered in the FKRPKD mice. Here we show that the laminin binding epitope of α-dystroglycan is present from the earliest stages of secondary myotube formation at E15.5 in wild type mice. FKRPKD mice show a mild reduction in laminin α2 at this time point. However, this reduction is not apparent using an antibody to pan laminin (identifies several laminin chains). However, at P0, a marked reduction of laminin α2 was noted in FKRPKD compared to wild type, together with a moderate reduction in the pan laminin, suggesting that the initial reduction in α2 subsequently alters the deposition of other laminin chains. In order to further examine this we have now examined the deposition of other laminin chains (α1, α4, α11, and Y1). There was no alteration in laminin α1, α4 and Y1 expression between wild type and mutant at either E15.5 or P0. Laminin α1 however, showed a subtle reduction in FKRPKD compared to wild type at E15.5 which became more apparent at P0. To determine the effect of these alterations on fibre formation we counted muscle fibres at E15.5 and P0 and showed that neither the number of primary myotubes (as indicated by slow myosin heavy chain expression) nor the total number of fibres at P0 was significantly different between FKRPKD and wild type mice. These observations suggest that alterations in basement membrane deposition during myogenesis do influence myoblast alignment and fusion.

http://dx.doi:10.1016/j.nmd.2013.06.509

P.8.9
Progressive muscular dystrophy in a mouse model of FKRP deficiency
M. Fernandez-Fuente1, C. Whitmore2, J. Kim2, C. Parr2, S.C. Brown2
1Royal Veterinary College, London, United Kingdom; 2Royal Veterinary College, Comparative Biomedical Sciences, London, United Kingdom

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 structural brain involvement, including Muscle Eye Brain Disease. The key defining feature of this group of disorders is the hypoglycosylation of α-dystroglycan and its inability to effectively bind extracellular matrix ligands such as laminin α2. We recently generated a mouse with a knock-down in FKRP in the muscle but not the central nervous system (FKRPKD). This mouse shows evidence of muscle fibre degeneration by 6 weeks of age. Despite the hypoglycosylation of α dystroglycan in all muscles examined, the soleus was only mildly affected relative to the other limb muscles. In order to investigate this aspect further we have now undertaken a detailed characterisation of the pattern of muscle involvement in this model, together with a study of the basement membrane changes that accompany the disease process. These analyses should assist in the design of future therapeutic strategies for the FKRP related group of diseases.

http://dx.doi:10.1016/j.nmd.2013.06.510

P.8.10
Losartan up-regulates NFκB signaling pathway and favors survival versus apoptosis in the dy2J/dy2J mouse model of Congenital Muscular Dystrophy
M. Elbaz1, N. Yanay1, S. Gelb1, M. Rabie2, S. Mitran Rosenbaum1, Y. Nevo1
1Hebrew University–Hadassah Medical School, Pediatric Neuromuscular Laboratory, Jerusalem, Israel; 2Hadassah, Hebrew University Medical Center, Neuropediatric Unit, Jerusalem, Israel, 3Hadassah, Hebrew University Medical Center, Goldyne Sadeva Institute of Gene Therapy, Jerusalem, Israel

Congenital Muscular Dystrophy (CMD) is a group of genetic disorders characterized by progressive loss of muscle strength and integrity. Merosin deficient congenital muscular dystrophy type 1A (MDC1A) is a common form of this disorder. Children affected with MDC1A suffer from early onset severe hypotonia and weakness with significant motor milestone delay. Often they do not achieve independent ambulation and die in the second or third decade. Despite extensive advances in diagnosis, cellular and molecular understanding, MDC1A remains a disease without a cure or any proven therapeutic option to relieve or slow disease progression. Our experimental data suggest that treatment with Losartan, an Angiotensin II type 1 receptor antagonist, results in significant clinical improvements and amelioration of fibrosis in the dy2J/dy2J mouse model of CMD through inhibition of TGFβ and MAPK signaling. We further examined Losartan’s effect on the cellular network, focusing on NFκB signaling. Previous studies suggested a role of NFκB in promoting muscle inflammation, necrosis and degeneration in Duchenne muscular dystrophy patients and animal models. Contrary to this, here we show that Losartan’s beneficial effect in the dy2J/dy2J of CMD is associated with NFκB signaling up-regulation manifested by enhanced serum TNFα level, decreased IκB-β protein level (NFκB inhibitor) and P65 accumulation in gastrocnemius nuclei of the dy2J/dy2J mice. A more in-depth investigation revealed that Losartan induced a modification in the NFκB gene expression towards pro-survival profile as cIAP2, TRAF2 and FTH mRNA levels were markedly increased following treatment. Losartan also induced the expression of anti apoptotic Bcl2 protein and down-regulated the expression of pro-apoptotic caspase 3 protein. Our study indicates that in the dy2J/dy2J mice of CMD, Losartan treatment resulted in NFκB activation with shifting from apoptosis/damage targeting pathway to a profile favoring cell survival.

http://dx.doi:10.1016/j.nmd.2013.06.511