CONGENITAL MYASTHENIA AND DYSFERLIN; ORAL PRESENTATIONS

G.O.4

Neuromuscular junction formation in Dok-7 deficient zebrafish embryos

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Congenital myasthenic syndromes (CMS) arise from genetic defects that affect transmission at the neuromuscular junction (NMJ). Causal mutations in presynaptic nerve terminal, synaptic cleft, and postsynaptic apparatus proteins have been identified. Recently, mutations in a novel CMS gene, *DOK* ("downstream of kinase") 7, were found to cause an autosomal recessive form of myasthenia. DOK7 mutations have emerged as one of the major genetic defects in CMS, accounting for about 10% of genetically diagnosed CMS cases. In contrast to other CMS subtypes, patients with *DOK7* mutations do not benefit from long-term therapy with esterase inhibitors. The precise function of the Dok-7 protein at the NMJ has not been elucidated, yet. Okada and co-workers generated mice lacking Dok-7 to explore its role in vivo. However, Dok-7 deficient mice were immobile at birth and died shortly thereafter. The zebrafish is an established model of vertebrate development and is also receiving increasing attention as a model of human disease; mutations that are lethal in mammals at early stages of development can be studied in the zebrafish. We therefore investigated the role of Dok-7 in endplate development and endplate maintenance in a zebrafish model. Downregulation of Dok-7 expression by injection of an antisense morpholino oligonucleotide into fertilised zebrafish eggs revealed first abnormalities of NMJ patterning in zebrafish embryos at 48 h post-fertilisation (hpf). By this time, motor axons in wild-type embryos have extended branches into and formed synapses with laterally located muscle fibres. Very early stages of NMJ formation do not seem to be affected in Dok-7 deficient embryos. These results might imply a role of Dok-7 in stabilising and branching of NMJs. Our results may help to determine the molecular mechanisms through which *DOK7* mutations compromise neuromuscular transmission in CMS patients. Furthermore, zebrafish may be a suitable model organism for testing novel treatments for patients with *DOK7* mutations.

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G.O.5

Partial functionality of a Mini-dysferlin molecule identified in a patient affected with moderately severe primary dysferlinopathy

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Primary dysferlinopathies are a heterogeneous group of autosomal recessive muscular dystrophies, caused by mutations in the large-sized DYSF gene encoding dysferlin. Dysfunction of dysferlin causes deficient sarcolemmal repair and leads to muscle fiber degeneration. In a patient presenting with moderately severe primary dysferlinopathy, we identified a homozygous multi-exonic internal deletion using genomic, transcriptional and F.I.S.H. analyses. Currently, this patient presents with proximo-distal weakness, but is still ambulant without a cane at 41 years. Bioinformatic analysis of putative Open-Reading-Frames within the resulting, deleted transcript predicts a possible translation into a truncated protein maintaining two C2 domains and the transmembrane domain. While muscle biopsy samples were not available, dysferlin protein analyses on monocytes obtained from the patient evidenced expression of a truncated molecule at the expected size, which localizes to the plasma membrane. Ectopic expression of the predicted Open-Reading-Frame using transfection into fibroblasts confirmed targeting to the plasma membrane. To further evaluate the functionality of this Mini-dysferlin, we constructed an AAV-vector containing the predicted Open-Reading-Frame identified in the patient under a muscle specific promoter. Intra-muscular injection of the Mini-DYSF-AAV vector in wild-type and dysferlin-deficient mice led to high-level expression of the truncated protein. Using a membrane repair assay, based on membrane wounding of transduced isolated muscle fibers with a two-photon laser-scanning microscope, we demonstrated that the Mini-dysferlin efficiently reseals the damaged sarcolemma. Therefore, this Mini-dysferlin is at least partially functional. Importantly, this demonstrates the modularity of dysferlin. In Duchenne muscular dystrophy, the identification of functional Mini-dystrophins led to the development of therapeutic strategies such as gene transfer of Mini-dystrophin and exon-skipping approaches. Our findings constitute a prerequisite for similar therapeutic strategies for a subset of primary dysferlinopathies (M.K., N.W., N.L., and I.R. contributed equally to this work).

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G.O.6

Sjl dystrophic mice express large amount of human muscle proteins following systemic delivery of human adipose-derived stem cells

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Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of disorders characterized by progressive degeneration of skeletal muscle caused by the absence or defective muscular proteins. The murine model for limb-girdle muscular dystrophy 2B (LGMD2B), the sjl mice, carry a deletion in the dysferlin gene that causes a reduction in the protein levels to 15% of normal. The mice show muscle weakness that begins at 4–6 weeks and is nearly complete by 8 months of age. The possibility to restore the defective muscle protein and improve muscular performance by cell therapy is a promising approach for the treatment of LGMD or other forms of progressive muscular dystrophies (PMD). Here we have injected human adipose stem cells (hASCs) in the sjl mice, without immunosuppression, aiming to assess their ability to: engraft into recipient dystrophic muscle after systemic delivery; form chimeric human/mouse muscle fibers; express human muscle proteins in the dystrophic host and improve muscular performance. We show for the first time that hASCs are not rejected after systemic injection even without immunosuppression, are able to fuse with the host muscle, express a significant amount of human muscle proteins and improve motor ability of injected animals. These results may have important applications for future therapy in patients with different forms of muscular dystrophies.

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APPLICATIONS OF ANIMAL MODELS OF DISEASE; POSTER PRESENTATIONS

G.P.8.01

Functional muscle, histological and biomarker analysis in the mdx mouse

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