Dissecting the pathogenesis of myofibrillar myopathies

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Myofibrillar myopathies (MFM) are inherited, progressive diseases of heart and skeletal muscle that often lead to severe physical impairment and premature death of affected patients. The knowledge about the precise mechanisms and signalling events that translate MFM causing gene mutations into the myopathic phenotype is limited, but critical for the development of targeted therapies. Therefore, this tandem research project (6 month stay in the lab of Dr. Sandri) aims on the establishment of zebrafish models and the elucidation of the pathophysiology and pathomechanisms of known MFM disease genes using state-of-the-art functional genomics approaches.

(1) MFM disease gene-deficient zebrafish (Morpholino-mediated knockdown) and transgenic zebrafish (Tol2-mediated transgenesis) overexpressing human MFM (cardio-) myopathy mutations in a heart and skeletal muscle-specific and inducible manner will be generated. The transgenic zebrafish lines will be evaluated in a wild-type but also disease gene-depleted background at different timepoints (embryo, larvae and adult). To generate stable null-background zebrafish, the genes will be knocked out using the TALEN technology.

(2) Depleted (Morphants and TALEN mutants) and transgenic zebrafish will be characterized functionally, structurally, molecularly and biomechanically. To do so, *in vivo* microscopy, histology and ultrastructural analysis, RNA-antisense in situ hybridization studies, qRT-PCR, Western Blots and immunostainings at the defined stages will be performed. In particular, probable effects on mitochondrial morphogenesis (e.g. transmission electron microscopy and MitoTracker Green FM stainings), the ubiquitin-proteasome system, and autophagy (e.g. Lc3-II conversion and LysoTracker staining) in cardiomyocytes and skeletal muscle cells will be evaluated.

(3) Finally, the PhD student will screen for novel muscle-specific interaction partners using Yeast-2-Hybrid (muscle library) and Tandem affinity purification (HL1 and C2C12 cells) assays. The *in vivo* role of the identified interactors, will be analyzed by reverse genetics approaches such as gene inactivation (Morpholino-knock-down) and over-expression studies (transient or stable) in zebrafish.