O6.2

Zebrafish as a model for dissecting the *in vivo* roles of Collagen VI

C. Consorti[1], V. Tonelotto[1], V. Trapani[1], N. Facchinello[1], P. Sabatelli[2], C. Giraudo[3], M. Cescon[1], C. Bertolucci[4], P. Bonaldo[1]

[1]Department of Molecular Medicine, University of Padova, Padova, Italy; [2]Institute of Molecular Genetics, National Research Council of Italy, Bologna, Italy; [3]Department of Medicine, University of Padova, Padova, Italy; [4]Department of Life sciences and Biotechnology, University of Ferrara, Ferrara, Italy

Chiara Consorti <chiara.consorti.1@phd.unipd.it>

Collagen VI (COL6) is a key extracellular matrix protein, expressed in different tissues where it is involved in a range of physiological and pathological processes. COL6 plays an important role in skeletal muscle, and mutations of COL6 genes are causative for different inherited muscle diseases in humans. To elucidate the pathogenic mechanisms underlying COL6-related disorders, diverse animal models were generated. However, the functions exerted by COL6 during embryogenesis remain to be elucidated. Thanks to its numerous advantages, zebrafish is a powerful model organism for studying vertebrate development and gene function. We generated a novel zebrafish COL6 null line through CRISPR/Cas9 site-specific inactivation of the *col6a1* gene. Phenotypic characterization of zebrafish COL6 null embryos and larvae revealed that lack of COL6 causes defective architecture of slow muscle fibers, with locomotor dysfunctions, together with defects in the motor axons elongation and in acetylcholine receptors patterning. Molecular and ultrastructural analysis showed that *col6a1* null fish display autophagy and organelle defects during development. Notably, treatment with salbutamol, a beta-2 adrenergic receptor agonist, leads to a significant amelioration of the neuromuscular defects in *col6a1* null embryos, thus paving the way for further drug studies in this animal model aimed at finding novel treatments for COL6-related disorders.

O6.3

Kbtbd13^{R408C}*-knockin* mouse model displays muscle-type dependent onset and progression of NEM6 myopathy

Ricardo Galli[1], Leon Beghtel[1], Shen Shengyi[2], Robbert van der Pijl[2], Henk Granzier[2], Josine de Winter[1], Coen Ottenheijm[1,2]

[1]Dept. of Physiology, Amsterdam University Medical Centers, Amsterdam, The Netherlands; [2]Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA

Ricardo Andres Galli <r.a.galli@amsterdamumc.nl>

Patients harboring mutations in *KBTBD13* (NEM6) display a impaired muscle relaxation, which compromises normal muscle function daily-life activities. The majority of NEM6 patients harbor the Dutch founder mutation (c.1222C>T, p.Arg408Cys). Recently, we identified that *KBTBD13*^{R408C} slows muscle relaxation through an actin-based mechanism. However, disease onset and progression remains largely uncharacterized. To study this, we characterized Soleus (SOL) and Extensor digitorium longus (EDL) morphology and function of homozygous *Kbtbd13*^{R408C}-knockin and wild type mice at 7 days, and 1, 3, 9 and 18 months of age. Morphological and functional assays of SOL and EDL of *Kbtbd13*^{R408C}-knockin mice showed no differences at 1 month old when compared to wild type mice. Nemaline bodies, slow-twitch fiber predominance, and hypertrophy of slow-twitch and fast-twitch fibers was observed in SOL muscle starting from 3-months-old and progressed over time. SOL intact muscle mechanics assays revealed impaired relaxation kinetics and decreased maximal tension starting at 3-months-old in *Kbtbd13*^{R408C}-knockin mice. Functional assays in EDL showed impaired maximal tetanic force and relaxation kinetics at 18-months-old in *Kbtbd13*^{R408C}-knockin mice, but not in younger mice.

In conclusion, the *Kbtbd13*^{R408C}-knockin mouse model phenocopies human NEM6 hallmarks, displaying a muscle-type dependent onset and disease progression, both structurally and functionally. Hence, this model enables us to further decipher the NEM6 pathomechanism and provides a therapeutic window to test treatment strategies.