
Revealing Altered T-Tubule Networks in Mtm1 Knockout Mice at 7 weeks through 3D myofibers Ultrastructural Imaging

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Résumé

Centronuclear myopathies (CNMs) are congenital disorders characterised by muscle weakness and the abnormal centralisation of nuclei within muscle fibers. The main genes associated with CNM include MTM1, DNM2, BIN1, RYR1, and SPEG. To date, no effective treatment is available. Despite extensive research, a limited understanding of the ultrastructural organisation of myofibers hampers our comprehension of CNM pathogenesis and the identification of potential therapeutic targets.

To overcome this limitation, we employed Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) imaging of skeletal muscle tissue from wild-type (WT) and Mtm1 knockout (KO) mice at different developmental stages (2 and 7 weeks). This approach provided high-resolution images, and we created a pipeline to segment mitochondria, transverse tubules and Z-disks, enabling the reconstruction of 3D skeletal muscle models.

The 3D reconstruction revealed a well-organised architecture of WT myofibers with regular sarcomeric alignments and an even distribution and shape of T-tubules and mitochondria, whereas for the Mtm1 KO mice, mitochondria and Z-disks seem normal, but T-tubules networks seems misoriented and more ramified. Furthermore, at 7 weeks of age, Mtm1 KO muscle displayed misoriented and ramified T-tubules, but also we noticed a variation in the Z-disks' orientation and sarcomeres delimitations. No major structural anomalies in mitochondria were visible, but further investigations are currently ongoing. To quantify the structural aberrations, we will next perform computational morphometry, and we plan to cross our findings with transcriptomic and proteomic data obtained at the same ages.

Overall, FIB-SEM analyses on Mtm1 KO muscle samples revealed the progressive appearance of architectural anomalies of the myofibers and especially of T-tubules, taking a central role in excitation-contraction coupling (ECC). Concomitantly, Mtm1 KO mice are normal at 2 weeks and exhibit severe muscle weakness at 7 weeks. T-tubule misalignment occurs as the first detectable anomaly, suggesting that it may serve as an early disease marker and potentially as principal therapeutic target.

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Mots-Clés: Centronuclear Myopathies, Myotubularin 1, Ultrastructural Imaging, Image Processing, Skeletal muscle, Quantification, 3D Reconstruction.