

Molecular mechanism of novel deletions in TPM3 that cause a hypercontractile phenotype with congenital muscle stiffness

Steven Marston¹, Maria Papadaki¹, Massimiliano Memo¹, Andrew Messer¹, Sandra Donkervoort², Carsten Bonneman², Kristen Nowak³, Royston Ong³, Elyshia McNamara³

¹Myocardial Function, Imperial College London, NHLI, London, W12 0NN, United Kingdom

²Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institutes of Health, Bethesda, MD, USA

³Harry Perkins Institute of Medical Research, University of Western Australia, Perth, Australia.

Patients with 'stiff child' syndrome usually have mutations at the interface of actin and tropomyosin that could affect the equilibrium of the Ca^{2+} -dependent switch of muscle. *ACTA1* K326N was previously reported and we now report two *de novo* TPM3 (Tpm3.12 protein) mutations, ΔE218 and ΔE224 , resulting in a significant hypercontractile phenotype with marked congenital muscle stiffness associated with ventilatory failure in one case.

The atomic resolution structure of tropomyosin bound to actin in the 'switched off' state shows that tropomyosin makes contact with actin at only two points: one of which is a cluster of basic amino acids: actin K326, K328 and R147. We have demonstrated that two Tpm2.2 mutations, ΔE139 and E181K, and the actin K326N mutation destabilize this actin-tropomyosin interface. We predicted that equivalent charge loss mutations at Tpm3.12 EE 218-219, EE 224-224, or ED 257-258 would also destabilise the interaction with actin leading to a partial switch-on of the muscle. The two newly discovered stiff patient mutations are located at two of the three predicted gain of function sites.

We used the quantitative *in vitro* motility assay and skeletal muscle thin filaments containing recombinant mutant Tpm3.12 expressed in a Baculovirus/sf9 system. ΔE218 led to a 2.5-fold increase in Ca^{2+} -sensitivity (EC_{50} ratio $\Delta\text{E218}/\text{WT} = 0.40 \pm 0.07$). ΔE224 also showed an increase in Ca^{2+} -sensitivity by 2.2-fold (EC_{50} ratio $\Delta\text{E224}/\text{WT} = 0.46 \pm 0.09$). It has been previously shown that there was a 2.5 fold increase in Ca^{2+} -sensitivity for the *ACTC* K326N mutation (EC_{50} ratio K326N/WT = 0.4 ± 0.05 , $p=0.07$).

The increased Ca^{2+} -sensitivity indicates that both mutations cause a gain of function that was predicted from the structural analysis and that can account for the stiff patient syndrome.