Molecular basis of stiff patient syndrome caused by mutations in *ACTA1* and *TPM3*

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3 of the 4 documented patients with stiff patient syndrome have mutations at the interface of actin and tropomyosin and therefore could affect the equilibrium of the Ca^{2+}-dependent switch of muscle. *ACTA1* gene (skeletal actin) K326N was previously reported, and we recently found stiff patients with ΔE218 and ΔE224 mutations in the *TPM3* gene (Tpm3.12 protein). 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. Tropomyosin has a seven-fold repeated structure corresponding to seven actin-binding interactions. We have demonstrated that two Tpm2.2 mutations, ΔE139 and E181K, and the actin K326N mutation destabilise the actin–tropomyosin interface. We predicted that equivalent charge loss mutations at 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. This was investigated by determining the Ca^{2+}-dependence of activation of motility in a single filament assay. Using the quantitative *in vitro* motility assay and skeletal muscle thin filaments containing recombinant mutant Tpm3.12, we found increased Ca^{2+}-sensitivity in both ΔE224 and ΔE218 tropomyosin mutations. ΔE218 led to a 2.5-fold increase in Ca^{2+}-sensitivity (EC_{50} ratio ΔE218/WT = 0.40 ± 0.07, p = 0.004). ΔE224 also showed an increase in Ca^{2+}-sensitivity by 2.2-fold (EC_{50} ratio ΔE224/WT for = 0.46 ± 0.09, p = 0.0). It has been previously shown that there was a 2.5-fold increase in Ca^{2+}-sensitivity for K326N actin (EC_{50} ratio K326N/WT = 0.4 ± 0.05, p = 0.07) in thin filaments reconstituted with wild type or mutant actin from biopsy and native tropomyosin and troponin. 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.