Figure 4.

Ca^{2+} control of *in vitro* motility. Representative Ca^{2+}-activation curves; ΔE218 left and ΔE224 on right compared with wild-type control. Thin filaments were assembled using baculovirus expressed Tpm3.12, rabbit skeletal muscle actin and rabbit skeletal muscle troponin. Thin filament movement over immobilized HMM was tracked and analyzed over a range of Ca^{2+} concentrations. Solid lines, control; dotted lines mutant tropomyosin. Error bars are SD for 4 measurements of motility in the same motility cell. The curves are the fits of the data to the Hill equation; sliding speed (top) and fraction of filaments motile parameters (bottom). Four separate Ca^{2+}-curves were determined for each mutation. For WT and ΔE218 EC_{50} for fraction motile were 0.20±0.06 and 0.07±0.02 respectively, for sliding speed 0.22±0.08 and 0.08±0.01 respectively. For WT and ΔE224 EC_{50} for fraction motile were 0.19±0.05 and 0.07±0.021 respectively, for sliding speed 0.30±0.08 and 0.09±0.02 respectively.

Figure 5.

A significant decrease in maximum force generation was seen in single fiber myofibres from Tpm3.12 ΔE224 & ΔE218 compared to controls (A). The Ca^{2+} sensitivity of force generation was increased in ΔE224 compared to controls and ΔE218 (B and C). Additionally there is reduced cross-bridge cycling kinetics in Tpm3.12 ΔE224 (D) while the active stiffness was not altered between Tpm3.12 ΔE224 and control (E).

Figure 6.

Location of gain-of function mutations in tropomyosin isoforms Tpm2.2 and Tpm3.12. The amino acids predicted to interact with actin are circled (A). Structure of the F-actin-tropomyosin complex determined by Li et al (2011). Surface rendering using PyMol. Actin K326, K328 and R147 are shown in different shades of blue and tropomyosin 218,219 223 and 224 are shown in magenta (B). The interface of tropomyosin period 6 with actin. Actin K326, K328 and R147 are shown in different shades of blue, E214 and E224 in red and 219 and 223 are shown in pink. Actin is represented by surface rendering; the secondary structure of tropomyosin backbone (coiled-coil alpha helix) is shown with the side chains in line notation (C).

Supplemental Video 1.

Gait of P1 at age 4 years. He walks with a stiff, shuffling gait, on his toes, with almost no flexion at the knees or hips.