Effects of myopathy-causing mutations R91P and R245G in TPM3 gene on structure and functions of slow skeletal muscle tropomyosin, its homo- and heterodimers

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Tropomyosin (Tpm) is one of the major proteins of the sarcomere, which binds actin and troponin and regulates muscle contraction. Tpm3.12 (γ-Tpm) is the main Tpm isoform in slow skeletal muscle. Mutations in Tpm3.12 among other disorders are known to cause congenital fiber type disproportion (CFTD), which is characterised by hypotrophy of slow muscle fibers, muscle weakness, troubles with walking and breathing. Still, little is known yet about molecular mechanisms of this disorder. In present work, we chose two mutations that are known to be associated with CFTD: Arg91Pro and Arg245Gly and applied various methods to investigate how these mutations affect structure and functions of Tpm. Mutations R91P and R245G both in γ*γ*- and γ*γ-Tpm homodimers (with mutations either in both or in only one of two γ-chains) as well as in γ*β-Tpm heterodimers (with mutation in the γ-chain) significantly destabilised Tpm complexes with F-actin, whose stability was measured by the temperature-dependent decrease in light scattering. Using differential scanning calorimetry, we showed that mutation R91P strongly destabilises N-terminal part of Tpm molecule by shifting its thermal transition to a lower temperature, while mutation R245G destabilises the C-terminal part of the molecule where it is located. The experiments in an *in vitro* motility assay were performed using myosin and troponin extracted from rabbit slow skeletal muscle. The results showed that the most dramatic decline in the sliding speed of regulated thin filaments occurred in γ*γ*-homodimers and γ*β-heterodimers; moreover, in that case the cooperativity of dependence of sliding speed from Ca concentration decreased, which means the impairment of regulation. In conclusion, our results showed that these myopathic mutations may cause significant disruption of Tpm structure and function, that is important for establishing the molecular mechanisms involved in CFTD development.

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