The sarcomeric α-actinins perform a dynamic balancing act at the muscle Z-line

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α-Actinin-3 is primarily expressed in fast (type 2) fibres in human skeletal muscle. A loss-of-function (LoF) polymorphism identified in the *ACTN3* gene (R577X) results in a shift in muscle function and performance. Complete α-actinin-3 deficiency (577XX) occurs in ~16% of humans worldwide and studies in elite, healthy and aged populations show this causes significant detriment to fast fibre function. We have developed an *Actn3* knockout (KO) mouse which mimics the human phenotype. Despite the total pool of Z-line α-actinins being maintained with an up regulation of α-actinin-2, KO mice display reduced grip strength, a shift towards slow/oxidative metabolism in fast fibres and increased calcineurin signalling. Over 50% of the human population have one copy of the *ACTN3* X allele, however heterozygosity (577RX) and dosage of α-actinin-3 has not been investigated. We assessed the relative impact of one copy of the *ACTN3* X allele by phenotyping *Actn3* heterozygous (HET) mouse. We hypothesized that HET mice would display an intermediate phenotype indicating a dosage effect associated with *Actn3* genotype. This included an intramuscular rescue and over-expression experiment with α-actinin-2 and -3 to test reciprocal regulation and the dose-response limits of the sarcomere pool. For the results analysis, all tissues from mouse experiments were excised after cervical dislocation. During the intramuscular rAAV experiments, mice were anaesthetized using 3.5% isoflurane in oxygen from a precision vaporizer and Buprenorphine was administered as an analgesic (0.01mg/kg) before the injection.

We can report that heterozygous mice are biologically intermediate compared to their WT and *Actn3* KO littermates. An additive gene-model can explain altered muscle mass, fast 2B fibre size, protein level of α-actinin-3, α-actinin-2 and downstream myofibrillar and metabolic proteins. Additionally we find post-natal delivery of α-actinin-3 into the *Actn3* KO mouse improves contractile force and alters recovery from fatigue. These contractile changes are accompanied with a dose-responsive reduction in α-actinin-2 and other Z-line proteins to demonstrate a delicate balance and endogenous reciprocal regulation of the sarcomeric α-actinins which directly influences function. Independent of the α-actinin isoform, overexpression disrupted the sarcomeric pool, resulting in muscle degeneration/regeneration to indicate a physiological threshold.

Our results reveal that the total pool of α-actinin proteins is tightly regulated and the reciprocal balance of α-actinin-2 and -3 in skeletal muscle can regulate metabolic, myofibrillar, and signalling proteins in a dose dependant manner. These findings provide insight into local muscle sarcomere α-actinin balance relevant to *ACTN3* genotype as well as individual responses to muscle disuse/disease and training in the human population.

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