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Human congenital myopathy actin mutants cause myopathy and alter Z-disc structure in *Drosophila* flight muscle

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Abstract

Over 190 mutations in the human skeletal muscle α-actin gene, *ACTA1* cause congenital actin myopathies. We transgenically expressed six different mutant actins, G15R, I136M, D154N, V163L, V163M and D292V in *Drosophila* indirect flight muscles and investigated their effects in flies that express one wild type and one mutant actin copy. All the flies were flightless, and the IFMs showed incomplete Z-discs, disorganised actin filaments and 'zebra bodies'. No differences in levels of sarcomeric protein expression were observed, but tropomodulin staining was somewhat disrupted in D164N, V163L, G15R and V163M heterozygotes. A single copy of D292V mutant actin rescued the hypercontractile phenotypes caused by TnI and TnT mutants, suggesting that the D292V mutation interferes with thin filament regulation. Our results show that expression of actin mutations homologous to those in humans in the indirect flight muscles of *Drosophila* disrupt sarcomere organisation, with somewhat similar phenotypes to those observed in humans. Using *Drosophila* to study actin mutations may help aid our understanding of congential myopathies caused by actin mutations.

Keywords: ACTA1; *Act88F*; *Drosophila*; IFM; Nemaline myopathy; Actin; Striated muscle; Muscle disease

1. Introduction

The contractile machinery of skeletal muscle consists of actin-containing thin filaments and myosin-containing thick filaments, precisely organised into repeating sarcomeric units. The optimal sarcomere length in working human skeletal muscle is 2.64 μm [1], with a thick filament length of 1.6 μm and a thin filament length of 1.27 μm. This precise organisation of actin and myosin in skeletal muscle is critical for optimal force generation. Thus, it is not surprising that mutations in the human skeletal muscle actin gene, ACTA1 result in skeletal muscle diseases (congenital myopathies) characterised by muscle weakness [2]. Mutations in ACTA1 cause nemaline myopathy (NM), as well as other myopathies with an overlapping phenotype, including intranuclear rod myopathy (IRM), actin filament aggregate myopathy (AM), congenital fibre type disproportion (CFTD) and congenital myopathy with core-like areas (reviewed in [3]). Mutations in 6 further genes including nebulin, (*NEB*, [4], troponin T, *TNNT1* [5], α-tropomyosin and β-tropomyosin, *TPM3* and *TPM2*, genes [6,7] cofilin-2 [8] and a member of the BTB/Kelch family [9] also cause NM.

Remarkably, over 190 mutations, occurring throughout the sequence in ACTA1, have been discovered [10], most of which are sporadic *de novo* dominant mutations. The different mutations result in one or more of a range of distinct subcellular phenotypes, of which nemaline rods in the sarcoplasm are most common. A subset of mutations causes

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actin rods to form within the myonuclei, and/or the accumulation of large aggregates of F-actin filaments, outside the sarcomeres. In a few cases the mutations result in congenital fibre type disproportion (CFTD), in which Type 1 (slow) fibres hypertrophy [11]. It is likely that most of the mutations in ACTA1 cause disease through dominant-negative effects rather than through reducing levels of expression [12,13]. However, there is still much to learn about how these mutations result in skeletal muscle disease.

Studying ACTA1 mutants *in vitro*, using cultured cells provides useful insights into their effects [12,14–16]. However, to understand fully how ACTA1 results in myopathy it is important to study NM actin mutations using an *in vivo* model. Model organisms such as zebrafish [17], *Drosophila* [18] and transgenic mice [19,20] as well as functional studies on mutations from biopsies (reviewed in [21]) have all been used successfully to investigate muscle diseases. In particular, *Drosophila* indirect flight muscles (IFMs) have a long history of being used successfully to investigate the effects of mutations in actin and other sarcomeric proteins on muscle function [22–25] .

Using *Drosophila* IFMs to investigate the effects of actin mutations has several distinct advantages. Of the six different actin genes in *Drosophila*, *Act88F* is the only sarcomeric isoform expressed in the IFMs [26,27]. Loss of *Act88F* expression only affects the IFMs, which are not required for fly survival, although *Act88F* is expressed in a few other adult muscles [25]. There is a high degree of homology (93% at the protein level) between human ACTA1 and *Drosophila* IFM-specific actin (*Act88F*). The IFMs are striated muscles with a very regular sarcomeric structure assembled from major muscle proteins similar to those in vertebrate skeletal muscle. Moreover, mutations can be introduced and studied in *Drosophila* relatively quickly and easily compared to generating transgenic mice.

Since *Drosophila* is a genetically tractable system we have used it to determine whether mutations in *Act88F* in the IFMs lead to the same or similar effects as observed in humans. We used P-element transformation to establish transgenic fly lines expressing one of six different site-directed *Act88F* mutations: G15R, I136M, D154N, V163L, V163M, D292V in the IFMs of *Drosophila*. These mutations were chosen because in humans they represent the spectrum of distinct phenotypes (Table 1 and see [28]). We undertook an in-depth analysis of their effects on muscle structure and function in both heterozygous and homozygous flies, to establish whether the *Drosophila* IFM is a good model system for studying the mechanisms leading to the distinct congenital actin myopathy phenotypes.

2. Materials and methods

2.1. Construction of pW8Act88F plasmids

In vitro mutagenesis was performed on a 4.0 kb *SalI* fragment of the *Act88F* gene excised from *pUC9Act88F* [22]. After subcloning into the $pP\{W8, w^{+mW.h}\}$ plasmid, a *Drosophila* germline trangenesis vector [29], between two *Xho*I restriction sites, the new plasmid, $pP\{W8, w^{+mW.hs}\ Act88F^{+}\}$ was sequenced and confirmed as wild type. The G15R, I136M, D154N, V163L, V163M and D292V mutations were introduced into $pP\{W8, w^{+mW.hs}\ Act88F^{+}\}$ using the QuickChange site-directed mutagenesis kit (Stratagene, UK) using mutant primers (Table 2). Mutated plasmids were then sequenced to verify that only the desired mutations had been introduced into the *Act88F* gene.

2.2. Fly genetics and transgenic Drosophila

Plasmid DNA for the wild type actin $pP\{W8, w^{+mW.hs}\ Act88F^{+}\}$, and six different actin mutants $pP\{W8, w^{+mW.hs}\ Act88F^{G15R}\}$, $pP\{W8, w^{+mW.hs}\ Act88F^{I136L}\}$, $pP\{W8, w^{+mW.hs}\ Act88F^{D154N}\}$, $pP\{W8, w^{+mW.hs}\ Act88F^{V163L}\}$, $pP\{W8, w^{+mW.hs}\ Act88F^{V163M}\}$ and $pP\{W8, w^{+mW.hs}\ Act88F^{D292V}\}$ was injected into y w^{1118}; Ki Δ2–3 *Drosophila* strain embryos following standard protocols. All the injections other than G15R and wild type actin were performed by Dr. Teresa Jagla, Myores *Drosophila* Transfection Platform, Clermont Ferrand, France. Transformants expressing the w^{+} (wild type eye colour) marker were recovered and at least two independent lines obtained for

Table 1
Comparison of Human and *Drosophila* phenotypes for each mutant heterozygote. This table shows each of the 6 different mutants, their classification in humans, showing the types of abnormalities observed, together with the features we observed for the same mutants in the IFMs of *Drosophila*.

Human biopsy		*Drosophila*
Mutation	Classification	Phenotype
I136M	Mild nemaline myopathy (NM)	Minor effects on muscle structure in heterozygotes. Zebra bodies only found in homozygotes. Small% of flies can fly.
D292V	Congenital fibre type disproportion (CFTD)	Some zebra bodies and disorganised muscle structure in heterozygotes. Homozygotes have relatively normal muscle structure.
G15R	Actinopathy (AM)	Large zebra bodies throughout muscle.
V163M	Actinopathy (AM) and intranuclear rods (IR)	Zebra bodies, Z-rings, Intranuclear Rods.
V163L	Severe actinopathy (AM) and intranuclear rods (IR)	Zebra bodies, Z-rings, Intranuclear Rods, muscle structure more disrupted than in V163M. Additional actin gene copy incompletely rescues phenotype.
D154N	Severe actinopathy (AM), nemaline myopathy (NM) and intranuclear rods (IR)	Zebra bodies, Z-rings, Intranuclear Rods, muscle structure more disrupted than in V163L. Additional actin gene copy incompletely rescues phenotype.