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Mutations and Polymorphisms of the Skeletal Muscle α-Actin Gene (*ACTA1*)

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Abstract

The *ACTA1* gene encodes skeletal muscle α-actin, which is the predominant actin isoform in the sarcomeric thin filaments of adult skeletal muscle and essential, along with myosin, for muscle contraction. *ACTA1* disease-causing mutations were first described in 1999, when a total of 15 mutations were known. In this article we describe 177 different disease-causing *ACTA1* mutations including 85 that have not been described before. *ACTA1* mutations result in five overlapping congenital myopathies: nemaline myopathy, intranuclear rod myopathy, actin filament aggregate myopathy, congenital fibre type disproportion and myopathy with core-like areas. Mixtures of these histopathological phenotypes may be seen in a single biopsy from one patient. Irrespective of the histopathology, the disease is frequently clinically severe, with many patients dying within the first year of life. Most mutations are dominant and most patients have *de novo* mutations not present in the peripheral blood DNA of either parent. Only 10% of mutations are recessive and they are genetic or functional null mutations. To aid molecular diagnosis and establishing genotype-phenotype correlations, we have developed a locus-specific database for *ACTA1* variations (http://waimr.uwa.edu.au/).

Keywords

skeletal muscle α-actin; *ACTA1*; congenital myopathies; locus-specific database

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BACKGROUND - ACTINS AND HUMAN DISEASE

Skeletal muscle α-actin, encoded by the *ACTA1* gene (MIM# 102610), is the principal actin isoform in adult skeletal muscle, forming the core of the thin filament of the sarcomere where it interacts with a wide array of proteins, notably myosin of the thick filament, producing the force for muscle contraction [Craig and Padrón, 2004].

Skeletal muscle α-actin is just one member of the actin protein family, which in humans consists of six isoforms that show > 90% similarity at the amino acid level [Sheterline, et al., 1998; Tondeleir, et al., 2009] (Supp. Figure S1). The actins are involved in many critical cell functions. β– and γ-actin form the intracellular actin cytoskeleton and are involved in cell morphology, cell motility and even in the nucleus [Sheterline, et al., 1998; Tondeleir, et al., 2009; Vandekerckhove and Weber, 1978]. The remaining four actin isoforms are expressed in smooth, cardiac or skeletal muscle where they are essential for contraction [Tondeleir, et al., 2009]. The two sarcomeric, striated muscle actins (skeletal muscle α-actin and cardiac α-actin), are co-expressed to varying degrees in skeletal and cardiac muscle [Ilkovski, et al., 2005; Tondeleir, et al., 2009].

Five of the six human actin isoforms have now been implicated in disease. These are the striated muscle actins skeletal muscle α-actin (*ACTA1*) [Kaindl, et al., 2004; Laing, et al., 2004; Nowak, et al., 1999] and cardiac α-actin (*ACTC1*) [Olson, et al., 1998; [Olson, et al., 2000], the cytoplasmic actins β-cytoskeletal actin (*ACTB*) [Nunoi, et al., 1999; Procaccio, et al., 2006] and γ-cytoskeletal actin (*ACTG1*) [van Wijk, et al., 2003; Zhu, et al., 2003] and smooth muscle α-actin (*ACTA2*) [Guo, et al., 2007]. The only human actin not yet associated with disease is γ-enteric actin (*ACTG2*). The mutations in skeletal muscle α-actin (*ACTA1*), the subject of this update, cause congenital myopathies: muscle diseases generally present at birth.

THE SKELETAL MUSCLE ALPHA-ACTIN GENE AND PROTEIN

The human *ACTA1* gene is located at 1q42.13. The *ACTA1* gene consists of seven exons, of which only six code for protein, and it extends over only 2.80 kbp of genomic DNA [Taylor, et al., 1988] (Figure 1). The *ACTA1* mRNA (NM_001100.3) codes for a 377 amino-acid nascent protein. The first two amino-acids are cleaved to produce the mature 375 amino-acid actin peptide, which is folded into a globular monomer protein, G-actin, that has a molecular weight of 42kDa [Sheterline, et al., 1998]. G-actin polymerises into filamentous or F-actin [Sheterline, et al., 1998]. The classic amino-acid numbering of the skeletal muscle α-actin molecule is based on the mature protein of 375 amino-acids, without the two N-terminal residues since this best matches the amino-acid sequence in all actins. However, this numbering of the amino-acid residues is not consistent with the guidelines of the Human Genome Variation Society (HGVS) of numbering the initiator methionine as amino-acid residue 1 and of numbering the cDNA sequence with the A of the initiator ATG as +1 (www.hgvs.org/mutnomen). In this article, *ACTA1* variations are numbered in the tables with both numbering systems, but in the text according to the HGVS guidelines.

The actins are a highly conserved protein family. At the amino-acid level, human skeletal muscle α-actin shows 100% homology with mouse skeletal muscle α-actin and 87% homology with rice cytoskeletal actin [Sheterline, et al., 1998] (Supp. Figure S1). Thus, most residues in skeletal muscle α-actin may be classified as very highly conserved, and any amino-acid change is likely to be disease-causing.

Although skeletal muscle α-actin is the predominant isoform in adult skeletal muscle, cardiac α-actin (the major actin isoform expressed in the adult heart) is the major actin

expressed in skeletal muscle during early gestation, but is downregulated late in the second trimester, and usually at negligible levels by birth[Ilkovski, et al., 2005]. Skeletal muscle α-actin and cardiac α-actin are 99% identical, differing at only 4 out of the 375 residues in the mature protein [Sheterline, et al., 1998].

VARIANTS IN *ACTA1*

Combining all available published reports with our unpublished data, we describe 177 different disease-causing variants in *ACTA1* (Figure 1, Supp. Table S1), 86 of which have not been previously reported. The 177 mutations comprise 157 missense, 5 nonsense, 5 frameshift and 4 splice site mutations, 1 in-frame duplication, 1 insertion of a single amino-acid, 1 in-frame single amino-acid deletion and 3 mutations of the normal stop codon. 133 of the mutations are “private”, being present in only one patient or family (Supp. Table S1, Supp. Figure S1). The 157 missense mutations affect 109 of the 377 (29%) amino-acid residues of skeletal muscle α-actin, with 134/157 or 85% occurring in residues conserved back to yeast (Supp. Figure S1). Thirty-three of the residues and the stop codon harbour more than one variant, while 55 of the amino-acid residues and the stop codon are mutated in multiple unrelated patients (Figure 1, Supp. Table S1, Supp. Figure S1). Only one mutation, pAla232Val, is also found amongst the known cardiac actin mutations.

Of the 177 mutations, 74 have been identified as arising *de novo*, while 21 show dominant and 17 show recessive inheritance (Supp. Table S1).

ACTA1 mutations are distributed throughout the six coding exons of the gene (Figure 1, Supp. Table S1). No mutations have as yet been found in the non-coding exon 1. The number of mutations in each exon correlates with the size of each exon (Table 1). Thus, there do not appear to be any particular mutation hotspots (Supp. Figure S1). There is a relative paucity of mutations between residues 301 and 334 (Supp. Figure S1). However, the corresponding region in cardiac α-actin (*ACTC1*) contains three of the nine mutations described in this gene (http://genetics.med.harvard.edu/~seidman/cg3/) (Supp. Figure S1), suggesting that mutations in this region of skeletal muscle α-actin will eventually be identified, perhaps in an as yet unrecognised disease phenotype.

Autosomal dominant mutations

The majority of *ACTA1* mutations identified in patients are sporadic, and thus there is no family history of the disease. Where DNA has been available for testing from the parents of sporadic patients, 79/94 or 84% had *de novo* dominant mutations not present in the peripheral blood DNA of either parent, while only 16% had recessive *ACTA1* mutations. There is therefore a high new mutation rate associated with *ACTA1* disease.

Stop codon mutations

Three sporadic mutations of the *ACTA1* stop codon have been found, with the stop codon replaced by glutamine, tryptophan or tyrosine and addition to the mature actin protein of 46 amino-acids translated from the 3’UTR [Wallefeld, et al., 2006]. The stop codon mutations were associated with a severe dominant phenotype in the three patients described [Wallefeld, et al., 2006].

Dominant inheritance

Although most *ACTA1* mutations are associated with isolated cases, and many affected patients do not reproduce, autosomal dominant transmission of *ACTA1*-related disorders may occur when an *ACTA1* mutation produces a mild phenotype compatible with adult life. This review includes 20 such variants, several of which have been published previously

(Supp Table S1). Although most families showing dominant inheritance display a relatively mild phenotype, intra-familial variation has been described in a number of families [Bouldin, et al., 2007; Nowak, et al., 1999; Ryan, et al., 2003].

There have been a number of instances where a severely affected proband was identified, and mildly affected or asymptomatic family members were found to carry the same dominant mutation. In at least three cases this can be explained by somatic mosaicism in one parent of a severely affected patient, demonstrated by the presence of only a small variant peak upon DNA sequencing, [Nowak, et al., 1999; Sparrow, et al., 2003] (Supp. Table S1). Disease severity would likely depend on the degree of mosaicism, the fraction and location of affected fibres and the proportion of mutant protein expressed in those fibres.

Autosomal recessive mutations

Recessive *ACTA1* disease is uncommon. Only 17 out of the 177 mutations, or 15 out of 259 families, reported here, are associated with recessive disease (Figure 1, Supp. Table S1).

Most recessive *ACTA1* mutations are nonsense, frameshift or splice-site mutations, and are predicted to cause premature termination of translation, or the omission of entire exons from the mRNA, leading to absence of skeletal α-actin protein [Agrawal, et al., 2004; Nowak, et al., 2007; Sparrow, et al., 2003]. Four missense mutations are associated with recessive disease, (p.His75Asn (His73Asn according to traditional numbering), p.Leu96Pro, p.Glu261Val and p.Met301Lys) (Figure 1, Supp. Table S1). All four of the recessive missense mutations are largely buried within the actin monomer (Figure 2). The recessive missense *ACTA1* mutations have been hypothesised [Sparrow, et al., 2003], and in the case of p.Leu96Pro and p.Glu261Val, demonstrated to be functional null mutations [Costa, et al., 2004]. All patients with recessive *ACTA1* disease therefore lack functional skeletal muscle α-actin

Heterozygous mutation carriers (parents or siblings of patients with recessive *ACTA1* mutations) were clinically unaffected, suggesting that expression from one intact copy of *ACTA1* is sufficient for normal function [Sparrow, et al., 2003]. Similar findings were observed in heterozygous skeletal muscle α-actin knock-out mice, which model human *ACTA1* recessive disease [Crawford, et al., 2002].

Intronic mutations

Five intronic mutations have been identified (Figure 1, Supp. Table S1). Four of these affect splice sites and cause recessive disease. But one intronic mutation results in dominant disease. This mutation, c.617-5C>A. is inferred by the splice site analysis algorithm at http://www.fruitfly.org/seq_tools/splice.html to create a cryptic splice site that leads to the insertion of a single additional alanine residue in the mature protein p.Ala206_Glu207insAla.

Variants showing reduced penetrance, and variants of uncertain effect

Three variants have been described that have been suggested to show incomplete penetrance. One frameshift mutation, p.Gly344AlafsX76, is predicted to result in translation of part of the 3'UTR to produce a larger protein [Agrawal, et al., 2004]. This mutation was associated with adult-onset disease in the proband, but no disease in the parent carrying the same variant, suggesting incomplete penetrance. All other frameshift mutations described to date lead to premature termination of the actin protein and have been associated with recessive disease. The misssense mutation p.Val136Ala was identified in a proband and the unaffected mother and grandmother (Agrawal, et al., 2004), while the heterozygous mutation p.Arg30Lys, was identified in both a proband and the possibly unaffected father.

There are also two variants of uncertain effect. The variants p.Lys70Lys and p.Glu209Asp were both observed as *de novo* variants in the one patient [Graziano, et al., 2004] (Table 2). The Lys70Lys mutation by *in vitro* analysis gave data suggesting it might affect splicing, but this could not be confirmed in patient muscle (Claudio Graziano unpublished observations). The p.Glu209Asp mutation is a minor change, perhaps unlikely to cause disease, though p.Glu207Asp has been reported in a proband and clinically unaffected father who had pathological changes on biopsy (Supp. Table S1). The pathogenicity of both the p.Lys70Lys and p.Glu209Asp mutations is thus currently uncertain.

Identification in further patients or families of these mutations and those demonstrating reduced penetrance may clarify their pathogenicity.

Polymorphisms in the *ACTA1* gene

Thirteen polymorphisms have been identified in the *ACTA1* gene to date (Table 3). These include the synonymous variations p.Thr108Thr, p.Thr151Thr, p.Ala183Ala and p.Ile332Ile and a number of intronic variations. No *ACTA1* missense variants have been classified as definite polymorphisms, though the final designation of those demonstrating incomplete penetrance or uncertain pathogenicity remains to be determined. It thus seems likely that most skeletal muscle α-actin amino-acid residues are critical for function, consistent with the very high conservation of the amino-acid sequence in all actins [Sheterline, et al., 1998] (Supp. Figure S1).

Graziano et al., (2004) [Graziano, et al., 2004] demonstrated that the 2 single nucleotide polymorphisms (SNPs) in intron 2, along with the cccgcc repeat element in intron 3 and the 3 SNPs in intron 5 form an extended haplotype.

Allele drop out

Primers that include SNPs can lead to preferential amplification of a single allele and allele drop out, resulting in disease-causing mutations going undetected. Graziano et al. 2004 [Graziano, et al., 2004] reported that allele drop out occurred with some of the primers described in the original paper by Nowak et al. (1999) [Nowak, et al., 1999] and used the primers listed by Ilkovski et al (2001) [Ilkovski, et al., 2001] to avoid intronic polymorphisms. A primer set that avoids the polymorphisms, and the associated protocols for their use are listed in Supp. Table S2.

ACTA1 LOCUS-SPECIFIC DATABASE

A locus-specific database has been established for *ACTA1* variations (http://waimr.uwa.edu.au/). The database contains all the variations listed in this update. The aims of the database are a) to make available information on all known mutations and polymorphisms in the *ACTA1* gene to laboratories testing for *ACTA1* mutations, and clinicians who have patients with *ACTA1* mutations and b) to provide information on the disease severity associated with mutations. To keep the database up-to-date and comprehensive, and therefore as useful as possible, any laboratory that identifies an *ACTA1* variant is urged to submit the information to the database.

CLINICAL RELEVANCE

Muscle diseases associated with *ACTA1* mutations

Mutations in *ACTA1* have to date been shown to cause five congenital myopathies: nemaline myopathy, intranuclear rod myopathy, and actin filament aggregate myopathy [Nowak, et al., 1999; Sparrow, et al., 2003], congenital myopathy with core-like areas [Kaindl, et al., 2004] and congenital fibre type disproportion (small, slow type I fibres) [Laing, et al., 2004]

(Figure 3). Any of these features in a muscle biopsy may thus indicate the cause is an *ACTA1* mutation. Mixtures of the pathological phenotypes may also be seen in one biopsy, for example congenital myopathy with both core-like areas and nemaline bodies [Jungbluth, et al., 2001] or actin aggregations, intranuclear rods and nemaline bodies [Schroder, et al., 2004].

Nemaline myopathy

Nemaline myopathy, first described in 1963 [Conen, et al., 1963; Schnell, et al., 2000; Shy, et al., 1963], is characterized histologically by the presence of sarcoplasmic nemaline bodies (rod-like structures) in affected muscles (Figure 3). Clinically, nemaline myopathy ranges in severity from a lack of spontaneous movement at birth, requiring immediate respiratory support, to mild disease and life well into adulthood [North, et al., 1997]. The European Neuromuscular Centre (ENMC) International Consortium has divided nemaline myopathy into six clinical subtypes: severe, intermediate, typical and mild (all congenital), adult onset, and other forms [Wallgren-Pettersson and Laing, 2000]. Adult onset nemaline myopathy is largely distinct from the congenital forms of the disease and frequently involves autoimmune components [Voermans, et al., 2008].

Congenital nemaline myopathy shows genetic heterogeneity, being to date known to be caused by mutations in six different genes, which all code for proteins associated with the muscle thin filament or actin. These genes do not account for all cases of nemaline myopathy, meaning there are other genes still to be identified [Jeannet, et al., 2007]. As well as skeletal muscle α-actin, the five other known genes are, in the order in which they were identified, slow α-tropomyosin (*TPM3*) [Laing, et al., 1995; Tan, et al., 1999; Wattanasirichaigoon, et al., 2002], nebulin (*NEB*) [Pelin, et al., 1999], slow troponin T (*TNNT1*) [Johnston, et al., 2000], β-tropomyosin (*TPM2*) [Donner, et al., 2002], and skeletal muscle specific cofilin-2 (*CFL2*) [Agrawal, et al., 2007]. Mutations in *TPM2, TPM3, TNNT1* and *CFL2* are rare causes of nemaline myopathy, with the majority of cases likely due to recessive mutations in *NEB* [Pelin, et al., 2002]. *ACTA1* mutations are the second most common cause of nemaline myopathy after mutations in nebulin, accounting for 20% to 30% of a cohort of nemaline myopathy patients [Agrawal, et al., 2004; Graziano, et al., 2004; Sparrow, et al., 2003] and are over-represented in the severe subtype [Agrawal, et al., 2004; Wallgren-Pettersson, et al., 2004b] with early death, though some *NEB* mutations also cause severe disease [Wallgren-Pettersson, et al., 2002]. The *ACTA1* mutations causing nemaline myopathy are distributed throughout most of the skeletal muscle α-actin molecule (Figure 2, Supp. Table S1).

Intranuclear rod myopathy

Intranuclear rod myopathy is a variant of nemaline myopathy characterised histologically by rod-like bodies in the nuclei of muscle fibres [Barohn, et al., 1994; Hutchinson, et al., 2006; Jenis, et al., 1969] (Figure 3) and is often, but not always, associated with a severe clinical phenotype [Hutchinson, et al., 2006; Kaimaktchiev, et al., 2006]. Currently 13 *ACTA1* amino-acid changes altering 12 different amino-acid residues are associated with intranuclear rod myopathy: p.His42Tyr, p.Gly57Arg, p.Gln139His, p.Ala140Pro, p.Leu144Phe, p.Thr150Ser, p.Asp156Asn, p.His163Asp, p.Val165Leu, p.Val165Met, p.Lys338Ile, p.Phe354Ser and p.Ile359Leu (Figure 2, Supp. Table S1). Mutations of Val165 (three different mutations at the genomic level, Supp. Table S1), are the most common intranuclear rod myopathy variant, having been reported in four independent patients or families to date [Hutchinson, et al., 2006; Kaimaktchiev, et al., 2006; Nowak, et al., 1999; Sparrow, et al., 2003; Weeks, et al., 2003].

Eight of the mutations causing intranuclear rod myopathy cluster between amino-acid residues 139 and 165, such that it is possible to predict to some extent where mutations causing intranuclear rod myopathy are most likely to be found.

Actin filament aggregate myopathy

Actin filament aggregate myopathy is characterized by the accumulation of actin-containing filaments in the muscle fibres [Bornemann, et al., 1996; Goebel, et al., 1997] (Figure 3) and is usually associated with severe disease. Eight different *ACTA1* mutations have been implicated in actin filament aggregate myopathy to date: p.Gly17Arg, p.Thr68Ile, p.Leu144Phe, pAlaSer146/7 duplication, p.Gly148Asp, p.Gly148Ser, p.Arg149Lys, p.Asp156Asn, p.Val165Leu. and p.Ser350Leu (Figure 2c, Supp. Table S1).

All reported mutations associated with intranuclear rod and actin aggregate myopathy have involved *ACTA1* [Hutchinson, et al., 2006; Laing and Nowak, 2005; Nowak, et al., 1999; Sparrow, et al., 2003]. However, not all patients display an apparent *ACTA1* mutation [Ryan, et al., 2003], suggesting that these disorders show genetic heterogeneity and/or that the screening protocols used did not detect all variants in *ACTA1*.

Similar to intranuclear rod myopathy, there is a hotspot of mutations associated with actin myopathy between residues 144 and 165, allowing prediction of where a mutation causing actin aggregate myopathy is most likely to be found. It is interesting that the hotspots for actin aggregate and intranuclear rod myopathies overlap and that the same mutation, for example p.Asp156Asn and p.Val165Leu, may cause both phenotypes.

Myopathy with core-like areas

Two mutations in *ACTA1*, p.Asp3Tyr, p.Glu336Lys, have been associated with congenital myopathy with core-like areas devoid of mitochondria/oxidative enzymes and not demonstrating any of the other pathologies (Figure 3). This disorder has been reported in two unrelated families and presents as mild, non-progressive skeletal muscle disease [Kaindl, et al., 2004]. Core myopathies display genetic heterogeneity, most often caused by mutations in the ryanodine receptor-1 gene (*RYR1*) [Robinson, et al., 2006], but also by mutations in selenoprotein N-1 (*SEPN1*) [Ferreiro, et al., 2002] and the myosin heavy chain-7 gene (*MYH7*) [Fananapazir, et al., 1993].

Both core-like areas and nemaline bodies associated with an *ACTA1* mutation (p.Met134Val) have been described in a patient presenting with relatively mild and non-progressive disease. Skeletal muscle from this patient displayed both nemaline bodies predominantly in type I fibres and core-like areas predominantly in type II fibres [Jungbluth, et al., 2001]. A mixed core-rod phenotype is more often caused by *RYR1* mutations [Monnier, et al., 2000; Scacheri, et al., 2000].

Congenital fibre type disproportion

Congenital fibre type disproportion (CFTD) is characterised by early onset, non-progressive generalized muscle weakness, where the primary histological abnormality is hypotrophy of type 1, slow twitch, muscle fibres with no other distinguishing features [Clarke and North, 2003; Laing, et al., 2004] (Figure 3). However, as fibre size disproportion is a feature of many other well-defined neuromuscular diseases, including other forms of congenital myopathy, these disorders must be excluded before a final diagnosis of CFTD can be made [Clarke and North, 2003; Laing, et al., 2004]. There are seven *ACTA1* mutations implicated in CFTD (p.Glu6Lys, p.Gly48Asp, p.Glu207Asp, p.Leu223Pro, p.Glu243Lys, p.Asp294Val, and p.Pro334Ser) (Figure 2, Supp. Table S1) although mutations in *TPM3* [Clarke, et al.,

2008] are a more common cause of CFTD and a recessive mutation in selenoprotein N1 (*SEPN1*) has also been identified [Clarke, et al., 2006].

Most of the *ACTA1* mutations known to cause congenital fibre type disproportion are located on one face of the actin monomer (Figure 2), suggesting the involvement of a specific actin binding protein or muscle function.

DIAGNOSTIC RELEVANCE

Patients with dominantly inherited nemaline myopathy are most likely to have mutations in *ACTA1*, while sporadic cases with no family history are most likely to have recessive mutations in *NEB* or *de novo* mutation of *ACTA1*. Since *ACTA1* is such a small gene and the rate of mutation detection is relatively high, it is advisable to start genetic screening of nemaline myopathy patients with *ACTA1*. Intranuclear rod myopathy and actin myopathy have to date only been associated with *ACTA1* mutations. *ACTA1* mutations are also a significant cause of congenital fibre type disproportion, but a rare cause of core myopathies. It is probable that other histopathologies, for example minimal change myopathy or non-specific myopathy [Kaindl, et al., 2004], may be associated with *ACTA1* mutations. Mutation analysis of the *ACTA1* gene is therefore warranted in any patient with a congenital myopathy, except those with central rather than internal nuclei.

The high proportion of *de novo* mutations causing dominant *ACTA1* disease should ensure that *ACTA1* disease is spread relatively evenly through all human populations. However, recessive *ACTA1* disease may be more prevalent in consanguineous communities, where the carrier frequency of one or more recessive mutations may be higher because of founder effects [Nowak, et al., 2007].

Few patients with *ACTA1* mutations show a cardiac phenotype [Ilkovski, et al., 2005; Ryan, et al., 2001], despite skeletal muscle α-actin accounting for around 20% of the striated muscle α-actin in the heart [Vandekerckhove, et al., 1986]. *ACTA1* disease patients who do have a cardiomyopathy have a hypertrophic cardiomyopathy eg [Kaindl, et al., 2004] which may be severe enough to be fatal [D'Amico, et al., 2006].

Demonstration of dominant or recessive *ACTA1* mutations elucidates the inheritance of the muscle disease in a family. Families of patients with *de novo ACTA1* mutations appear recessive, with no family history and neither parent affected, but an affected child. Identifying a dominant *de novo ACTA1* mutation thus clarifies the genetic cause and mode of inheritance. It also changes the recurrence risk from the 1:4 for recessive disease, to the undefined risk of germline mosaicism. The parents of an affected patient with a *de novo ACTA1* mutation should be counselled as possibly having a germline *ACTA1* mutation.

The fact that the *ACTA1* mutations show some level of consistency in the disease caused and in disease severity (Supp. Table S1) allows a level of prognosis. The accuracy of prognosis should continue to increase as further mutations are identified and known mutations are identified in additional patients.

All recessive *ACTA1* patients studied show retention of cardiac actin in all skeletal muscle fibres. Immunohistochemistry for cardiac actin is therefore warranted for severely affected congenital myopathy patients.

BIOLOGICAL RELEVANCE

Skeletal muscle α-actin structure and function

The actin monomer is a globular protein (G-actin) that binds ATP and one divalent cation (Mg^{2+} *in vivo*). The monomer is divided into two domains of equal size by a cleft containing the bound nucleotide and cation (Figure 2). The two domains are connected by two strands of the polypeptide chain, the “hinge”, that allows intramolecular movement. Each of the two domains is then further divided into subdomains 1 and 2; and 3 and 4 [Sparrow, et al., 2003]. Filamentous actin (F-actin) is formed by polymerisation of G-actin and is a helical filament where subdomains 3 and 4 of each monomer are close to the axis and subdomains 1 and 2 are on the outer edge [Craig and Padrón, 2004]. Each actin monomer within F-actin has a number of intermolecular contacts with its four neighbouring monomers [Sheterline, et al., 1998;Sparrow, et al., 2003]. In skeletal muscle, F-actin polymerised from skeletal muscle α-actin forms the core of the sarcomeric thin filament, and interacts with the other thin filament proteins including nebulin, tropomyosins and troponins. Skeletal muscle α-actin also interacts with many other proteins for example in the Z-disc, and importantly with myosins in the thick filament. Muscle contraction is achieved when calcium binds to the troponin complex, causing a conformation change and the movement of tropomyosin across the surface of the skeletal muscle F-actin. This movement exposes the myosin binding sites on skeletal muscle α-actin, allowing actin-myosin interaction and muscle contraction [Craig and Padrón, 2004]. Skeletal muscle α-actin therefore has many binding partners and functions, any of which may be affected by disease-causing mutations.

Based on the finding that recessive mutations of *ACTA1* are null alleles, it is likely that the functional consequences of dominant *ACTA1* mutations stem from dominant negative effects rather than an absence of actin function. That is, the mutant allele produces a “poison peptide” because the mutant G-actin monomer interacts with wild-type monomers, is incorporated into F-actin, and subsequently interferes with the function, assembly and stability of the thin filaments. The dominant *ACTA1* mutations may interfere with any of the normal functions of skeletal muscle α-actin. The experimental evidence to date, recently reviewed by Feng and Marston (2009) [Feng and Marston, 2009], indicates that different measurable properties of G- or F-actin are affected by the different *ACTA1* mutations, but there are no clear correlations between the functions affected, or the location of the mutations within known binding sites and the five histopathological phenotypes. This is in contrast to the clear distinction found between the effects of sarcomeric protein mutations that cause dilated or hypertrophic cardiomyopathy. Mutations that cause dilated cardiomyopathy decrease calcium sensitivity and usually decrease cross-bridge turnover, whereas those that cause hypertrophic cardiomyopathy always increase calcium sensitivity and usually increase cross-bridge turnover [Feng and Marston, 2009]. The *ACTA1* mutations associated with congenital myopathies increase or decrease a) polymerisation into F-actin, b) the sliding speed of actin filaments, c) Ca2+ regulation, or d) the strength of binding to α-actinin [Feng and Marston, 2009]. Each *ACTA1* mutation may affect multiple actin functions [Feng and Marston, 2009]. The congenital fibre type disproportion (CFTD) mutation p.Glu294Val showed one of the few clear-cut effects, in locking tropomyosin in the “off” position on the actin filament [Clarke, et al., 2007]. This and other data led Clarke et al (2007) [Clarke, et al., 2007] to hypothesise that the main effect of *ACTA1* mutations causing congenital fibre type disproportion was on sarcomere function while the effect of those causing the other histopathologies were on sarcomere structure [Clarke, et al., 2007].

It has been hypothesised that the *ACTA1* mutations associated with intranuclear rods interfere with the nuclear export signals in the actin monomer thus leading to the accumulation of skeletal muscle α-actin in the nucleus [Ilkovski, et al., 2004] or cause increased trafficking of the mutant skeletal muscle α-actin into the nucleus [Domazetovska,

et al., 2007a]. It was noted in the patient described by Kaimaktchiev et al [Kaimaktchiev, et al., 2006] that the number of intranuclear rods increased in a subsequent biopsy while the disease had not progressed, indicating intranuclear rods did not necessarily mean a poor prognosis. Domazetovska and colleagues hypothesise that sequestration of mutant skeletal muscle α-actin in intranuclear rods and other aggregates may in fact spare the patient sarcomeres and result in a milder disease [Domazetovska, et al., 2007b].

As previously stated, one of the most clear-cut effects of *ACTA1* mutations on actin biology is that the recessive missense mutations prevent normal folding of the actin monomer, leading to a functionally null protein [Costa, et al., 2004].

The *ACTA1* mutations causing the various histopathologies in patients tend to cause the same phenotype in tissue culture studies, with mutations causing intranuclear rods or sarcoplasmic rods in patients also forming these structures in culture [Domazetovska, et al., 2007a; Ilkovski, et al., 2004]. This is not always consistent. For example, the p.Arg185Gly mutation formed intranuclear rods in culture while they were not seen in patient biopsies [Ilkovski, et al., 2004]. This non-concordance may result from a sampling problem with the patient muscle biopsy, since it is well known that nemaline bodies may not be seen in all biopsies [North, 2004; Ryan, et al., 2003]. It has also been argued that the artificial situations of tissue culture or *in vitro* analysis may lead to different effects to those seen in patients [Feng and Marston, 2009]. This may also apply to mouse models of *ACTA1* disease. The *Acta1* knock-out mouse model relatively accurately models human recessive *ACTA1* disease [Crawford, et al., 2002], but the p. His42Tyr knock-in mouse model unexpectedly shows a more severe phenotype in male than female mice [Nguyen and Hardeman, 2008], though it has never been suggested that male nemaline myopathy patients are more severely affected than female patients.

Remarkably, it seems, that despite decades of research we simply do not know enough about actin structure-function correlations to be able to properly interrogate the *ACTA1* mutations [Feng and Marston, 2009].

Whatever the pathological effect of the mutations, it appears that for many there is a dosage effect, with the level of the mutant protein controlling the disease severity in the patient. This is especially clear from the mildly affected somatic mosaic parents of severely affected children. The lack of heart problems in almost all *ACTA1*-disease patients, is further evidence of a dosage effect of *ACTA1* mutations, since around 20% of the striated muscle α-actin in a normal heart is skeletal muscle α-actin [Vandekerckhove, et al., 1986]. Interestingly, extraocular muscle function (as in most other patients with nemaline myopathy) is spared in *ACTA1* patients and it has recently been demonstrated that extraocular muscles express similar levels of cardiac α-actin to the heart [Ravenscroft, et al., 2008]. One might expect that some *ACTA1* mutations would have a deleterious effect even at very low levels of expression, perhaps also therefore in the heart if they, for example, act as thin filament end-capping mutants [Sparrow, et al., 2003]. The p.His42Tyr mouse model gives evidence that low levels of mutant protein are sufficient to cause pathology [Nguyen and Hardeman, 2008]. The p.Lys338Glu mutation associated with fatal cardiomyopathy, when tested *in vitro*, was shown to decrease the strength of interaction with α-actin in 10-fold [D'Amico, et al., 2006].

The patients with no skeletal muscle α-actin due to recessive mutations have nemaline bodies [Nowak, et al., 2007], yet, there is no mutant protein produced in these patients' muscles. These patients have cardiac α-actin in their skeletal muscles rather than skeletal muscle α-actin, but the cardiac α-actin is not mutant. This means that nemaline bodies can form in the absence of mutant protein. Consistent with this, nemaline myopathy also results

from absence of slow α-tropomyosin [Tan, et al., 1999], β-tropomyosin [Monnier, et al., 2009] and slow troponin T [Johnston, et al., 2000]. Nemaline bodies may therefore result from a change in the normal stoichiometry of sarcomeric proteins, as well as the presence of a mutant sarcomeric protein.

Genotype – phenotype correlations

Within the cohort of patients with dominant *ACTA1* mutations the relationship between genotype and phenotype may be examined by comparing: a) the mutations leading to the pathologically distinct diseases; b) the clinical presentation of individuals within families showing dominant inheritance and c) the phenotype of unrelated patients who share the same mutation.

As previously mentioned, 55 amino acid residues in *ACTA1*, plus the stop codon, are mutated in multiple unrelated patients (Supp. Table S1, Supp. Figure S1), with many mutated residues showing some phenotypic stability. One of the clearest associations is between phenotype and mutations of p.Val165 (to leucine or methionine), which have been associated with intranuclear rod pathology in three sporadic cases and one dominant family (Supp. Table S1). Other mutations which have resulted in a similar phenotype in multiple patients include p.His75Leu/Arg (severe nemaline myopathy; 2 patients), pAsn117Thr (mild nemaline myopathy; 2 patients), p.Met134Val (mild nemaline myopathy; 2 patients). However, in the case of the mutation with the largest number of patients identified to date (p.Gly270Cys; 8 patients) the phenotype is highly variable, ranging from mild to severe. This might suggest that as more patients are identified with each of the mutations the spread in phenotypes for each mutation will increase.

At present there are perhaps simply too few patients identified with each of the dominant *ACTA1* mutations to make many clear-cut inferences about genotype-phenotype correlations. As more patients are identified that share mutations, the relationship (or lack of) between genotype and phenotype may become more apparent. However, the variation in disease severity between unrelated patients with the same mutation, along with intra-familial differences, suggests an important role for as yet undefined epigenetic or environmental factors that modify the phenotype of *ACTA1* congenital myopathies.

It is easier to reconcile genotype with phenotype in patients carrying recessive *ACTA1* mutations than in patients with the more numerous dominant missense mutations. Patients with recessive *ACTA1* disease are devoid of skeletal muscle α-actin, but cardiac α-actin expression is maintained, and the level of cardiac α-actin determines the clinical severity [Nowak, et al., 2007]. Patients lacking skeletal muscle α-actin but with sufficient upregulation of cardiac α-actin may have milder disease than patients with one dominant *ACTA1* mutation [Nowak, et al., 2007].

FUTURE PROSPECTS

The most significant future challenge is to develop effective therapies for the *ACTA1* diseases [Nowak, 2008]. Individuals suffering from severe *ACTA1*-related disease often die in infancy or may survive but be confined to a wheelchair, requiring constant respiratory support and tube feeding. Thus, any therapy providing even a small to moderate improvement in muscle function would be helpful. Currently, there are limited therapeutic options for patients affected with *ACTA1*-associated diseases. There has been a report of some clinical improvement in nemaline myopathy patients taking tyrosine supplements [Ryan, et al., 2008], but most treatment options are aimed at symptomatic relief and prophylaxis of complications. This includes respiratory assistance, which is of enormous benefit, not least in terms of quality of life [Wallgren-Pettersson, et al., 2004a].

The *ACTA1* mutations affect many actin functions. Thus, therapies aimed at individual functions are unlikely to be effective for a majority of patients and the therapy of choice would therefore be one that is independent of actin function [Feng and Marston, 2009; Nowak, 2008]. It has been concluded that some of the experimental therapies being investigated for Duchenne muscular dystrophy, where most effort has been directed, will not work for *ACTA1* diseases [Nowak, 2008]. For example, antisense-induced exon skipping is unlikely to work for *ACTA1* diseases, since there are only six coding exons and all appear essential for function. The effect of factors that cause muscle hypertrophy, insulin-like growth factor 1 (IGF-1) and four and a half LIM domain protein 1 (FHL1), has been investigated in the p.His42Tyr knock-in mouse model with suggestions of positive results [Nguyen and Hardeman, 2008].

Functional striated muscle actin is essential for muscle contraction [Jaeger, et al., 2009]. It has therefore been proposed that one way to treat *ACTA1* disease might be to replace the mutant skeletal muscle α–actin by uregulating cardiac α–actin, the 99% identical fetal skeletal muscle actin isoform [Nowak, 2008]. In the recessive *ACTA1* patients, those with the higher levels of cardiac α-actin function best. Increasing the levels of cardiac α-actin even more may thus further improve muscle function in these patients. It has now been demonstrated that transgenic expression of high levels of cardiac α-actin can rescue the skeletal muscle α-actin knock out mice that normally die by nine days postnatal, so that they live to adulthood with remarkably normal muscle function [Nowak, et al., 2009]. The question becomes whether this can be applied to human patients. Considerable research over many years has been carried out into the factors controlling expression of cardiac and skeletal muscle α–actin including suggestion, amongst other things, that the myogenic regulatory factor MRF4 may be involved in the differential expression of the two genes [Moss, et al., 1996]. However a recent review concluded that still little is known about the regulation of expression of the different actin isoforms [Tondeleir, et al., 2009].

Whether upregulation of cardiac α-actin in patients with dominant *ACTA1* mutations would show a similar benefit is unknown. However, the mild disease in somatic mosaic parents and general lack of effect of *ACTA1* mutations in the heart and extraocular muscles would suggest that decreasing the proportion of mutant actin might be effective for at least some of the dominant mutations.

CONCLUSION

ACTA1 mutations are numerous, mostly *de novo* dominant and private to individual patients. They are a significant cause of severe congenital myopathies with currently no curative treatment. Many laboratories around the world now offer a diagnostic service for *ACTA1*. This article and the associated locus-specific database should aid diagnosis of the *ACTA1* diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Linear diagram of the *ACTA1* gene showing the six coding exons and the location of all mutations. The dominant mutations are in normal text, the recessive mutations are italicised and at the bottom of the list for each exon. The open boxes represent translated regions of each exon, the filled boxes the 5' and 3' untranslated regions. See Supp. Table S1 for detailed list of mutations, including the DNA mutation names.

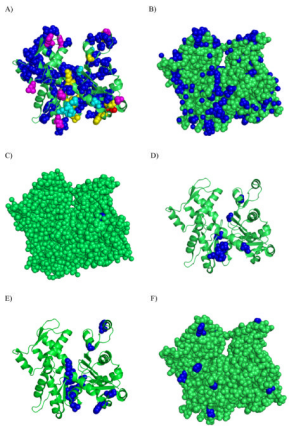

Figure 2.

Location of *ACTA1* mutations within the actin monomer. The figure was generated with Polyview (http://polyview.cchmc.org/polyview3d.html) based on the Protein Database file 1j6z which does not have locations for the 3 most N-terminal and 3 most C-terminal amino-acids of the mature skeletal muscle α–actin protein. A number of the *ACTA1* mutations therefore cannot be placed in the figure, including one of the two core myopathy mutations. A) All mutations colour-coded ribbon diagram. Nemaline myopathy: blue, actin myopathy: cyan, intranuclear rod myopathy: yellow, core myopathy: red, congenital fibre type disproportion: magenta. B) All mutations space fill. C) Recessive nemaline myopathy missense mutations. D) Actin myopathy. E) Intranuclear rod myopathy. F) Congenital fibre type disproportion.

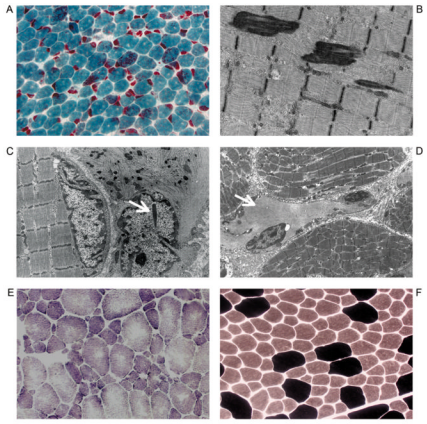

Figure 3.

Light and electron microscopic images of the five different histopathologies caused by *ACTA1* mutations. A) Light microscopic gomori trichrome stain of nemaline myopathy. B) electron micrograph of nemaline bodies in nemaline myopathy. C) Electron micrograph of an intranuclear rod (arrowed) in intranuclear rod myopathy. D) Electron micrograph of accumulation of actin thin filaments (arrowed) in actin aggregate myopathy. E) Light microscopy of core like areas (NADH staining) in core myopathy. F) Light microscopy (ATP staining pH 10.4) showing small (pale) slow (Type 1) muscle fibres and large (dark) fast (Type 2) muscle fibres in congenital fibre type disproportion. A B, D, E and F courtesy of Caroline Sewry,

TABLE 1

Distribution of *ACTA1* mutations

	Number of coded amino-acids (% of total)	Dominant mutations	Recessive mutations	Total	Percentage of total
Exon 1 (non-coding)	0	0	0	0	0%
Intron 1		0	0	0	0%
Exon 2	43 (11)	12	1	13	7%
Intron 2		0	0	0	0%
Exon 3	108.3 (29)	49	5	54	31%
Intron 3		0	1	1	1 %
Exon 4	54 (14)	28	2	30	17%
Intron 4		1	1	2	1%
Exon 5	64 (17)	31	3	34	19%
Intron 5		0	1	1	1 %
Exon 6	60.6 (16)	14	1	15	8 %
Intron 6		0	1	1	1 %
Exon 7	47 (12)	25	1	26	15 %
Total		160	17	177	

Table 2

ACTA1 variants of uncertain pathogenicity

Exon	DNA Variation[a]	Effect on Protein (traditional numbering in brackets)	Disease Phenotype	Reference	Remarks	Inheritance
3	c.210G>A	p.Lys70Lys (Lys68Lys)	NMT	[Graziano, et al., 2004]	This patient had two *de novo* mutations: Lys70Lys and Glu209Asp. The Lys70Lys mutation by *in vitro* analysis gave data suggesting it might affect splicing. This could not be confirmed in patient muscle. The pathogenicity of both mutations is therefore currently uncertain.	*de novo*
5	c.627G>C	p.Glu209Asp (Glu207Asp)	NMT	[Graziano, et al., 2004]		*de novo*

[a]Nucleotide numbering reflects cDNA numbering (*ACTA1* cDNA RefSeq: NM_001100.3.) with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. Traditional numbering of amino acids reflects the amino-acid residues in the mature actin protein following the removal of the two N-terminal amino-acids. The sequence variation descriptions were checked using the Mutalyzer program [Wildeman, et al., 2008] using RefSeq NM_001100.3 for exonic mutations and RefSeq AF182035.1 for intronic mutations.

Table 3

Polymorphisms in *ACTA1*

Exon/Intron	DNA Variation	Effect on Protein	Effect on Protein (traditional amino-acid numbering)	Remarks	Reference(s)
Exon 1	c.-66C>T			Known polymorphism: rs605428	
Exon 1	c.-65T>C			Known polymorphism: rs605430	
Intron 2	c.130-10G>C			Known polymorphism: rs41271481	[Graziano, et al., 2004]
Intron 2	c.130-5T>C			Known polymorphism: rs11803533	[Graziano, et al., 2004]
Exon 3	c.324C>A	p.Thr108Thr	Thr106Thr	Known polymorphism: rs.41271479	
Exon 3	c.453C>A	p.Thr108Thr	Thr149Thr	Known polymorphism	[Mayosi, et al., 1999]
Intron 3	c.454+*29CCCGCC(3_5)			Known polymorphism	[Graziano, et al., 2004; Nowak, et al., 1999]
Intron 3	c.455-53C>A			Known polymorphism: rs527621	
Exon 4	c.549G>A	p.Ala183Ala	Ala181Ala		This report
Intron 5	c.809-35delG			Known polymorphism: rs.59228224	[Graziano, et al., 2004]
Intron 5	c.809-14G>C			Known polymorphism: rs6673359	[Graziano, et al., 2004]
Intron 5	c.809-12dupC			Known polymorphism	[Graziano, et al., 2004]
Exon 7	c.996C>A	p.Ile332Ile	Ile330Ile	Known polymorphism	[Mayosi, et al., 1999]

ACTA1 cDNA RefSeq: NM_001100.3. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. Traditional numbering of amino acids reflects the amino-acid residues in the mature actin protein following the removal of the two N-terminal amino-acids. The sequence variation descriptions were checked using the Mutalyzer program [Wildeman, et al., 2008] using RefSeq NM_001100.3 for exonic mutations and RefSeq AF182035.1 for intronic mutations.