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Review

Muscle disease caused by mutations in the skeletal muscle alpha-actin gene (*ACTA1*)

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Abstract

Mutations in the skeletal muscle alpha-actin gene (*ACTA1*) associated with congenital myopathy with excess of thin myofilaments, nemaline myopathy and intranuclear rod myopathy were first described in 1999. At that time, only 15 different missense mutations were known in *ACTA1*. More than 60 mutations have now been identified. This review analyses this larger spectrum of mutations in *ACTA1*. It investigates the molecular consequences of the mutations found to date, provides a framework for genotype–phenotype correlation and suggests future studies in light of results of investigation of normal and mutant actin in other systems, notably the actin specific to the indirect flight muscles of *Drosophila*. The larger series confirms that the majority of *ACTA1* mutations are dominant, a small number are recessive and most isolated cases with no previous family history have de novo dominant mutations. The severity of the disease caused ranges from lack of spontaneous movements at birth requiring immediate mechanical ventilation, to mild disease compatible with life to adulthood. Overall, the mutations within *ACTA1* are randomly distributed throughout the protein. However, the larger series of mutations now available indicates that there may be clustering of mutations associated with some phenotypes, e.g. actin myopathy. This would suggest that interference with certain actin functions may be more associated with certain phenotypes, though the exact pathophysiology of the actin mutations remains unknown.

Keywords: Mutations; Skeletal muscle; Myofilaments

1. Introduction

ACTA1 gene mutations have been shown to cause three different congenital myopathies [1].

(i) The first of these is 'actin myopathy' (AM), a term first applied by Goebel et al. [2] for a disease phenotype described previously [3,4] in which patients' biopsies reveal homogeneous filamentous inclusions containing actin, occupying areas devoid of sarcomeres, which would normally be part of the myofibrillar filament lattice (Fig. 1, Table 1).

(ii) The second is intranuclear rod myopathy (IRM) with characteristic intranuclear inclusions [2,5] (Fig. 1, Table 1).

(iii) The third, but commonest, disease is nemaline myopathy (NEM) with characteristic sarcoplasmic nemaline bodies (rods) [6,7] (Fig. 1, Table 1).

More than one phenotype may be caused by one *ACTA1* mutation [1] (Table 2, Fig. 2). Further *ACTA1* gene mutations are continually being identified in patients with the three conditions. Over 60 *ACTA1* mutations are

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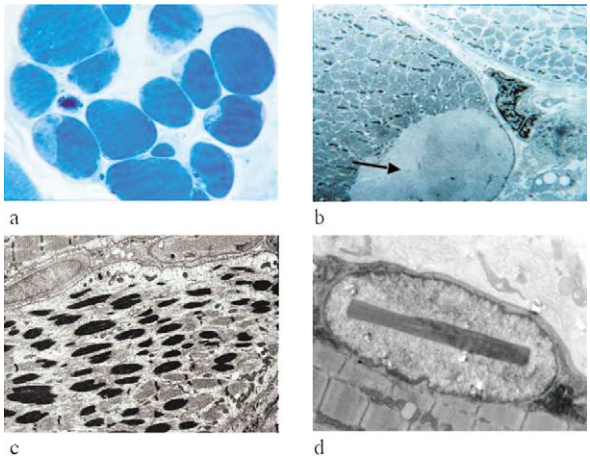

Fig. 1. (a) Actin myopathy (Val163Leu)—light microscopy of semi-thin section showing pale areas characteristic of the actin accumulations (courtesy of Hans Goebel). (b) Actin myopathy (Val163Leu)—electron microscopy. Arrow points to accumulation of actin filaments (courtesy of Hans Goebel). (c) Nemaline myopathy caused by an *ACTA1* mutation (Met132Val) (courtesy of Caroline Sewry). (d) Intranuclear rod myopathy caused by an *ACTA1* mutation (Val163Met) (courtesy of David Hutchinson).

therefore now known (Table 2, Fig. 2). In addition to the nemaline and intranuclear rods and actin accumulations, *ACTA1* mutations also effect changes in size and distribution of muscle fibre types.

AM and IRM are rare, but NEM is a more common and better known congenital myopathy [13]. NEM ranges in severity from a paucity of spontaneous movements at birth requiring immediate mechanical ventilation, to mild disease compatible with life to adulthood [14]. The European Neuromuscular Centre (ENMC) International Consortium on Nemaline Myopathy has divided NEM into six different subtypes: severe, intermediate, typical, mild, adult onset and other forms based on the severity of the disease, age of onset and additional features [15] (Table 1). In addition to *ACTA1*mutations [1,9,10,12], NEM has been associated with mutations in four other genes coding for muscle thin filament proteins. These are: (1) slow α-tropomyosin (*TPM3*) [16,17,18]; (2) nebulin (*NEB*) [19]; (3) slow troponin T (*TNNT1*) [20]; and (4) β-tropomyosin (*TPM2*) [21]. In addition, mutations in the skeletal muscle ryanodine receptor gene (*RYR1*) can cause nemaline bodies as well as central cores in the mixed muscle disorder core–rod myopathy [22,23]. Linkage analysis indicates that *NEB* mutations cause the majority of NEM cases. *ACTA1* mutations appear to be the second most common cause of NEM [8] at around 20% of cases. The small size of the actin gene makes it easier to find the *ACTA1* NEM mutations than those in the giant *NEB* gene, so more disease-causing mutations are known in *ACTA1*, with many *NEB* mutations remaining unidentified.

Mutations in human actin genes, including the cardiac actin gene, *ACTC*, which produce dilated [24] or hypertrophic [25,26] cardiomyopathy are a relatively recent discovery. In *Drosophila melanogaster*, the fruitfly, mutants of the *Act88F* muscle actin gene, whose expression is largely restricted to flight muscles and which is not therefore required for organism viability, have been known for some time [27,28]. Mutations in this animal model have clarified the molecular functions of actin and its dysfunction in muscle development and can aid our understanding of human actin mutations.

In this review we will concentrate on 69 characterised *ACTA1* gene mutations (Table 2, Fig. 2) with a view to developing an understanding of genotype–phenotype correlations and suggesting future studies. Mutations are distributed through all six coding exons of *ACTA1* (Fig. 2). There is as yet no overlap between the mutant *ACTA1* residues that produce skeletal myopathies and those in *ACTC* that cause cardiomyopathies, despite the proteins being 99% identical [29].

2. The genetics of *ACTA1* mutations

ACTA1 mutations cause both dominant and recessive disease, with all of the sporadic cases for whom parental DNA has been available having de novo dominant

Table 1
Muscle phenotypes caused by skeletal muscle alpha actin gene mutations

Disease	Abbreviation	Histopathological phenotype[a]	Clinical phenotype	Genetics
Actin myopathy	AM	Accumulation of actin filaments in muscle fibres	Usually severe congenital muscle weakness and high mortality	AD, de novo mutations
Intra-nuclear rod myopathy	IRM	Rod bodies in muscle nuclei	Usually severe congenital muscle weakness and high mortality	AD, de novo mutations
Nemaline myopathy	NEM	Sarcoplasmic rod bodies	Highly variable from severe congenital muscle weakness and high early mortality to mild disease compatible with life well into adulthood. Nemaline myopathy subtypes: severe, intermediate, typical and mild, adult onset, other.	AD/AR, de novo mutations

[a] The different histopathological phenotypes may co-exist in the one patient.

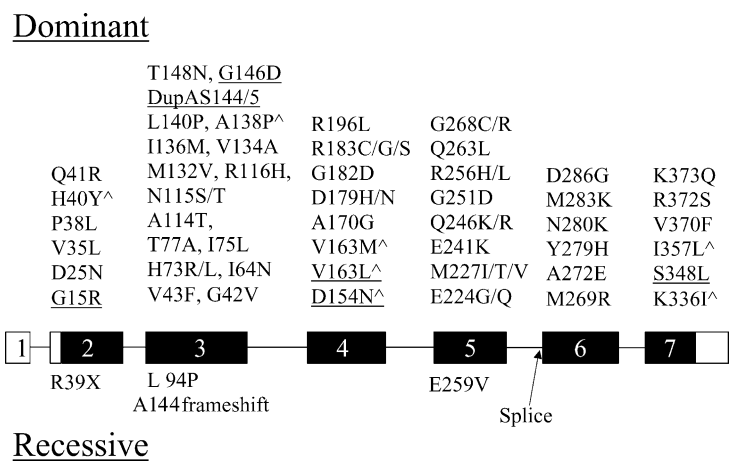

Fig. 2. Distribution of mutations in the different exons of *ACTA1*. Coding exons in black, non-coding exons in white. Dominant mutations are listed above the gene diagram, recessive below. Actin myopathy mutations are underlined, ^ = intranuclear rods, the remainder = nemaline myopathy.

mutations not present in either parent. Sixty mutations are associated with NEM, seven with AM and eight with IRM. Some mutations are associated with more than one type of muscle pathology, e.g. Val163Leu with both AM and IRM (Table 2, Fig. 2). In two instances, Val163Leu and Met227Ile, two different DNA mutations cause the same amino acid change.

There are 65 missense mutations, one frameshift, one nonsense, one duplication and one splice-site mutation. Of the missense mutations, 24 arose as de novo dominant mutations, 12 show dominant inheritance, while only two, Leu94Pro and Glu259Val, are recessive. Fifteen *ACTA1* codons have to date shown multiple mutations. Twelve of the amino acid variations (His40Tyr, Gln41Arg, Asn115Thr, Met132Val, Val163Leu, Arg183Gly, Met227Ile, Met227Val, Glu259Val, Gly268Arg, Gly268Cys, and Lys373Gln) have recurred in different families. Disease severity tends to be similar in different families with the same mutation, but not always. Genetic and environmental modifying factors may alter the severity of the disease and perhaps even the histopathological phenotype. A tropomyosin modifier (suppressor) of troponin mutants has been identified in *Drosophila* [30] and four *Act88F* mutants act as suppressors of the same troponin mutants (Haigh and Sparrow, unpublished) but to date, no modifiers of actin mutants have been identified in this system.

Ten of the actin amino acid residues have also been mutated to more than one other amino acid (His73, Asn115, Val163, Asp179, Arg183, Glu224, Met227, Gln246, Arg256, and Gly268; (Table 2, Fig. 2). We do not know whether these residues are especially mutable or serve particularly crucial functions in actin. However, as no polymorphic amino acid variants have been identified among hundreds of normal *ACTA1*alleles sequenced, it seems that most actin residues are critical for function, in accordance with actin's high evolutionary conservation. *ACTA1*gene polymorphisms identified include only silent polymorphisms at Thr149 and Ile330 and intronic polymorphisms.

The frameshift and nonsense mutations probably produce null alleles as described below. The duplication may increase the size of an important part of the actin protein. The splice site mutation was identified in a family showing recessive inheritance, with both unaffected parents heterozygotes and three severely affected children homozygotes. The effects of this mutation on *ACTA1* mRNA and protein are not yet known.

ACTA1 mutations have not been identified in a number of cases diagnosed histologically as AM or IRM. Clearly, either the screening methods used are missing some *ACTA1* mutations, or mutations in other genes can produce these conditions, or both.

3. The pathophysiology of *ACTA1* mutations

3.1. Actin

Actin is found in all cells and forms, as polymerised F-actin, a major part of the cytoskeleton. Within muscle cells, actin, myosin and their associated proteins form the specialised contractile structure known as the sarcomere. The roles of actin in the cytoskeleton and sarcomere require interactions with a great variety of proteins. These have likely restrained actin evolution, probably explaining why it is one of the most conserved proteins known [29].

The actin monomer is a globular protein (G-actin). It is a slow ATPase and binds one divalent cation; in vivo this is Mg^{2+}. Structurally the molecule is divided into two domains of equivalent size by a cleft containing the bound nucleotide and cation (Fig. 3). The domains are connected by only two strands of the single polypeptide chain, known as the 'hinge', which allows intramolecular movement. Each domain is further subdivided into subdomains (1 and 2)

Table 2
Skeletal muscle alpha-actin gene (*ACTA1*) mutations

Exon	Amino acid change[a]	Mutation	gDNA[b]	Phenotype NEM	AM	IRM	Age at death	Inheritance	Origin	Reference[c]
Mutations—coding										
2	Gly15Arg	GGC-CGC	1728G > C		+		3 months	De novo	H. Goebel/J. Anderson	[1,2]
2	Asp25Asn	GAC-AAC	1758G > A	Severe			2 months	Isolated case	N Romero	
2	Val35Leu	GTG-CTG	1788G > C	Severe			Alive at 1.5 years	De novo	A. Beggs	
2	Pro38Leu	CCC-CTC	1798C > T	Severe			15 days	De novo	A. Beggs	
2	Arg39STOP	CGA-TGA	1800C > T	Severe			22 months (ventilation from birth)	Recessive	N Romero	
2	His40Tyr	CAC-TAC	1803C > T	Severe		+	2 months	De novo	A. Beggs	[1]
	His40Tyr	CAC-TAC	1803C > T	Typical		+	Alive at 42 years	Isolated case	C. Wallgren-Pettersson	
2	Gln41Arg	CAG-CGG	1807G > A	Typical			Unknown	De novo: twins	C. Graziano	[8]
	Gln41Arg	CAG-CGG	1807G > A	Typical			Alive at 21, 54 years	Dominant,	A. Beggs	
2	Gly42Val	GGC-GTC	1916G > T	Mild			Alive at 47 years	Dominant	G. Matthijs	
3	Val43Phe	GTC-TTC	1918G > T	Severe			2.5 months	De novo	J. Hertz	
3	Ile64Asn	ATC-ACC	1982T > C	Typical			Alive at 7, 48 years	De novo,	A. Beggs	
3	His73Arg	CAC-CGC	2009A > G	Intermediate			Alive at 8 years	Isolated case	A. Beggs	
3	His73Leu	CAC-CTC	2009A > T	Severe			5 days	De novo,	A. Beggs	
3	Ile75Leu	ATC-CTC	2014A > C	Severe			Alive at 1.8 years	Unknown	A. Beggs	
3	Thr77Ala	ACC-GCC	2020A > G	Typical			Alive at 4 years	De novo,	A. Beggs	
3	Leu94Pro	CTT-CCT	2072T > C	Severe			5 and 19 days (with Glu259Val)	Recessive	R. Sutphen	[1]
3	Ala114Thr	GCC-ACC	2131G > A	Severe			5 months	Isolated case	C. Wallgren-Pettersson	
3	Asn115Ser	AAC-AGC	2135A > G	Typical			Alive at 33, 18 3 years	Dominant	K North	[1,9]
3	Asn115Thr	AAC-ACC	2135A > C	Mild			Alive at 42 years	Dominant	C. Wallgren-Pettersson	
	Asn115Thr	AAC-ACC	2135A > C	Mild			Alive at 31 years	Isolated case	L. Dalpra/ C. Wallgren-Pettersson	
3	Arg116His	CGC-CAC	2138G > A	Severe			1 day	De novo	E. Honey,	
3	Met132Val	ATG-GTG	2185A > G	Typical			Alive at 39 years	Isolated case	F Muntoni/ C. Wallgren-Pettersson	[1,10]
3	Met132Val	ATG-GTG	2185A > G	Mild			Alive at 9 years	Isolated case	M. Molina/ C. Wallgren-Pettersson	
3	Val134Ala	GTG-GCG	2192T > C	Typical			Alive at 3 years	Dominant ?incompletely penetrant in mother and grandfather	A. Beggs	
3	Ile136Met	ATC-ATG	2199C > G	Typical			Alive at 45 years	Isolated case	K North	[9]
3	Ala138Pro	GCC-CCC	2203G > C	Severe		+	Alive at 18 months	Isolated case	N Romero	
3	Leu140Pro	CTG-CCG	2210T > C	Typical			Alive at 10, 35 years	Dominant	N Romero	
3	A144 frameshift deletion	GCC-CC	2221delG	Severe (with Glu259Val)			2 months (with Glu259Val)	Recessive	A. Beggs	
3	Two amino acid duplication AlaSer144/5	Duplication of six bp (GCCTCC)	2221–2226 dup		+		5 months	Isolated case	H. Schmalbruch,	[4]
3	Gly146Asp	GGC-GAC	2228G > A		+		6 months	Dominant-	A. Beggs	
3	Thr148Asn	ACC-AAC	2234C > A	Mild			Alive at 50, 30 years	Dominant,	N Romero	
4	Asp154Asn	GAC-AAC	2375G > A	+	+	+	2 months	Isolated case	J.M. Schroeder	
4	Val163Leu	GTG-CTG	2402G > C		+	+	Alive at 7.5 years	De novo	H. Goebel, C. Hubner	[1,2]
4	Val163Leu	GTG-TTG	2402G > T		+	+	4 months	Isolated case	H. Goebel.	[1,2]
4	Val163Met	GTG-ATG	2402G > A	+		+	Alive at 64, 34, 33, and 3 years	Dominant	D. Hutchinson	
4	Ala170Gly	GCG-GGG	2424C > G	Severe			16 years	Isolated case	K. Bushby/ C. Wallgren-Pettersson	
4	Asp179Asn	GAC-AAC	2450G > A	Mild			Alive at 34 years	Isolated case	N. Romero	
4	Asp179His	GAC-CAC	2450G > C	Mild			Alive at 44 years	Isolated case	N. Romero	
4	Gly182Asp	GGC-GAC	2460G > A	Typical (intermediate)			Alive at 3 years	De novo,	A. Beggs	[1]
4	Arg183Cys	CGC-TGC	2462C > T	Severe			1 and 4 days	Dominant: Father mosaic	J. Vigneron	[1]

and (3 and 4). Polymerisation of G-actin to form filamentous, F-actin, proceeds more rapidly at one end of the filament (+, or barbed end) by addition of the ATP-G-actin monomer. After monomer addition, ATP hydrolysis occurs, producing a conformational change that is important for F-actin stability and function. F-actin is a helical filament in which all the monomers are oriented similarly, with subdomains 3 and 4 close to the filament axis and 1 and 2 on the outer edge. Each actin monomer makes specific interactions with four neighbouring monomers.

In striated muscle sarcomeres, F-actin forms the core of the thin filament, which also contains nebulin, tropomyosin and the troponin complex. Cyclical interactions between actin and myosin (located in the muscle thick filaments), which consume ATP, cause muscle contraction. A major role of the thin filaments is thus to transfer the forces developed by myosin to the Z-disc and thereby serially throughout the myofibril. Importantly, striated muscle activation occurs by the binding of calcium to the troponin C polypeptide of the troponin complex. Upon calcium binding, the troponin C and associated troponin I undergo conformational changes releasing the troponin I from binding actin and permitting the movement of the troponin complex (troponins C, I

Table 2 (*continued*)

Exon	Amino acid change[a]	Mutation	gDNA[b]	Phenotype NEM	AM	IRM	Age at death	Inheritance	Origin	Reference[c]
4	Arg183Gly	CGC-GGC	2462C > G	Severe			13 months	De novo	K. North	[9]
	Arg183Gly	CGC-GGC	2462C > G	Intermediate			Died at 1 year	De novo	A. Beggs	
4	Arg183Ser	CGC-AGC	2462C > A	Severe			Unknown	Isolated case,	N. Romero	
4	Arg196Leu	CGT-CTT	2502G > T	Severe			14 days	Isolated case	W. Kress	
5	Glu224Gln	GAG-CAG	2668G > C	Severe			3 years	Isolated case,	I. Nonaka	
5	Glu224Gly	GAG-GGG	2669A > G	Typical			Alive at 3 years	De novo	Beggs	
5	Met227Val	ATG-GTG	2677A > G	Typical			Alive at 3 years	De novo,	A. Beggs	
	Met227Val	ATG-GTG	2677A > G	Typical			Alive at 7 years	Isolated case	N. Goemans/ C. Wallgren-Pettersson	
5	Met227Thr	ATG-ACG	2678T > C	Severe			14 days	De novo	C. von Kaisenberg	
5	Met227Ile	ATG-ATA	2679G > A	Typical			Alive at 31, 58 years	Dominant	J. Giltay/ C. Wallgren-Pettersson	
5	Met227Ile	ATG-ATC	2679G > C	Typical			Alive at 39 years	Isolated case	A. Beggs	
5	Glu241Lys	GAG-AAG	2719G > A	Severe			Alive at 7 years	De novo	I. Nonaka	
5	Gln246Lys	CAG-AAG	2734C > A	Mild			Alive at 32 years	Isolated case	A. Clark/ C. Wallgren-Pettersson	
5	Gln246Arg	CAG-CGG	2735A > G	Mild			Alive at 8, 14, 16, 39, 40 years	Dominant,	A. Beggs	[11]
5	Gly251Asp	GGC-GAC	2750G > A	Intermediate			Alive at 8 years	De novo	Beggs	
5	Arg256His	CGC-CAC	2765G > A	Severe			Alive at 10 years	De novo	C. Wallgren-Pettersson	[1]
5	Arg256Leu	CGC-CTC	2765G > T	Severe			3 years 9 months	Isolated case	I. Nonaka	
5	Glu259Val	GAG-GTG	2774A > T	Severe			5 and 19 days (with Leu94Pro)	Recessive	R. Sutphen	[1]
	Glu259Val	GAG-GTG	2774A > T	Severe			2 months (with frameshift mutation)	Recessive	A. Beggs	[8]
5	Gln263Leu	CAG-CTG	2786A > T	Severe			Alive at 21 months	De novo	C. Müller/ C. Wallgren-Pettersson	[1,12]
5	Gly268Arg	GGT-CGT	2800G > C	Severe			Alive at 5 years	Isolated case,	I Nonaka	
	Gly268Arg	GGT-CGT	2800G > C	Severe			Unknown	Isolated case	N. Romero	
5	Gly268Cys	GGT-TGT	2800G > T	Mild			Alive at 10 years	De novo	K. North	[9]
	Gly268Cys	GGT-TGT	2800G > T	Intermediate			Alive at 7 years	Isolated case	A. Beggs	
6	Met269Arg	ATG-AGG	2895T > G	Mild			Alive at 27 years	De novo	F. Muntoni/ C. Wallgren-Pettersson	
6	Ala272Glu	GCG-GAG	2904C > A	Intermediate			5.5 months	Isolated case	J. McGaughran	
6	Tyr279His	TAC-CAC	2924T > C	Intermediate			9 months	De novo	A Beggs	
6	Asn280Lys	AAC-AAG	2929C > G	Severe			9 months	De novo	A. Beggs	[1]
6	Met283Lys	ATG-AAG	2937T > A	Typical			Alive at 6 years	De novo	H. Jungbluth/ C. Wallgren-Pettersson	
6	Asp286Gly	GAC-GGC	2946A > G	Severe			9 months	Isolated case,	A. Beggs	[1]
7	Lys336Ile	AAA-ATA	3174A > T	Not classified		+	Alive at 2.5y	Isolated case,	D. Chitayat/ C. Wallgren-Pettersson	
7	Ser348Leu	TCG-TTG	3210C > T		+		Alive at 10y	De novo	Yoram Nevo,	
7	Ile357Leu	ATC-CTC	3236A > C	Severe		+	6 months	De novo	K. North	[9]
7	Val370Phe	GTC-TTC	3275G > T	Severe			Alive at 4 months	De novo	I. Hughes/ C. Wallgren-Pettersson	[1]
7	Arg372Ser	CGC-AGC	3281C > A	Severe			6 months	Dominant: mother mosaic	T Hansen/ C. Wallgren-Pettersson	
7	Lys373Gln	AAA-CAA	3284A > C	Mild			Unknown	Isolated case	C. Graziano	[8]
	Lys373Gln	AAA-CAA	3284A > C	Typical			Alive at 7, 34 years	Dominant,	A. Beggs	
Mutations, non-coding										
	Splice-site mutation Intron 5/exon 6	ag/GTAT-at/GTAT	2891G > T	Severe			1 m, 10 m	Recessive	N. Goemans/ C. Wallgren-Pettersson	

[a] All mutations were heterozygous except the recessive Arg39STOP and the splice site mutation.

[b] Variations numbered according to Genbank AF182035.

[c] All mutations without a reference are unpublished.

and T) and tropomyosin (Tpm) across the F-actin surface, thereby exposing the myosin binding sites and activating contraction [31].

3.2. The effects of functional null alleles of the ACTA1 gene

A recessive *ACTA1*mutant producing a severe NEM phenotype is due to a nonsense mutation of codon 39 (R39X), a premature translation termination signal. A similar mutation, *KM88* (or T79X) in the *D. melanogaster Act88F* actin gene causes a dominant flightless phenotype with severe sarcomeric abnormalities [32]. *KM88* flight muscles show no evidence that the N-terminal peptide, if produced, accumulates. The messenger RNA appears to be degraded [32], an effect well known as nonsense-mediated mRNA decay [33]. If the R39X mutation does behave similarly to *KM88*, it is likely no polypeptide is produced; but even if it were, it would likely be inactive, containing only small parts of subdomains 1 and 2.

The recessive nature of R39X provides two important insights into *ACTA1* functional genetics. First, it suggests that one wild type *ACTA1* gene copy is sufficient for normal function. Second, it argues that the dominance of the vast majority of *ACTA1* mutations is not due to an absence of all actin function, but to dominant negative effects. In other words, the mutant alleles produce ‘toxic’ polypeptides that

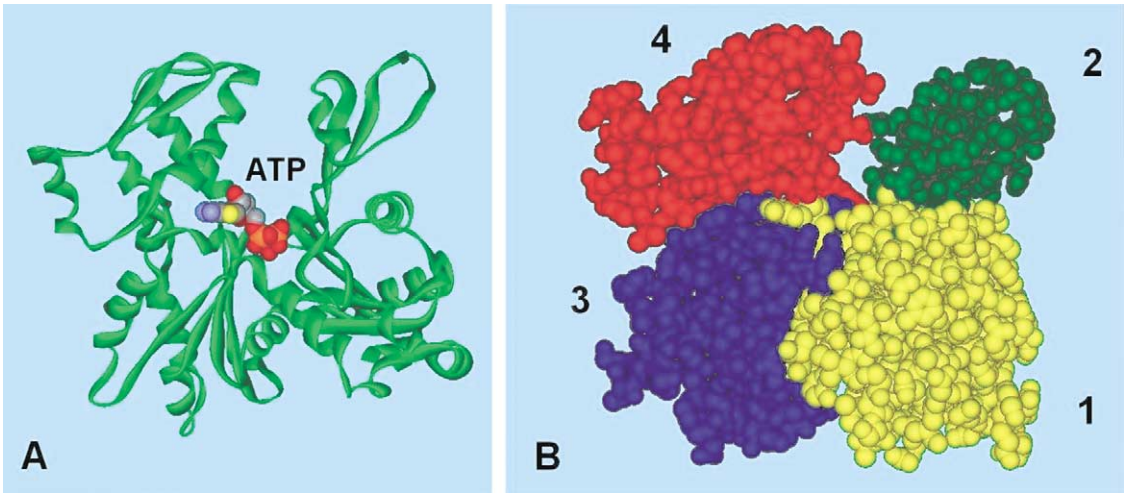

Fig. 3. Atomic structure of actin. (A) In ribbon form with ATP shown in molecular form within the cleft. (B) Structure coloured by domain; domain 1 and ATP—yellow; domain 2—green, domain 3—blue and domain 4—red. Molecular structure generated using Accelerys ViewerLite software (http://www.accelerys.com) and the 1ATN.pdb file.

interfere with the activity of wild-type actin generated by the non-mutant gene copy.

Support for this proposal comes from another recessive *ACTA1* allele with a NEM phenotype, A144delG, a frameshift mutation caused by a base deletion in codon 144. The polypeptide will contain only the N-terminal 143 actin residues followed by a 145-residue unrelated sequence ending at codon 188. This polypeptide may not be formed due to mRNA instability (see above), but if it were, would contain a complete actin subdomain 2 and most of subdomain 1 minus residues 338–375. In F-actin, subdomains 1 and 2 contribute actin–actin intermolecular contacts [34], so this mutant could act in a dominant negative fashion, but this is unlikely.

A similar conclusion can be drawn from *ACTA1* knockout mice [35]. Hemizygous null mice are viable and overtly normal, but the null homozygotes, while appearing normal at birth, die by 9 days from weakness and starvation [35]. Upregulation of cardiac actin, normally expressed in developing foetal skeletal muscle, and vascular actin, occurs in the muscles of both the heterozygous and hemizygous null mice and probably explains the survival of the null mice for a few days after birth [35].

Dominant negative or antimorphic actin mutants have been described in *Drosophila* [27] and yeast [36,37] where experimental genetics allows determination of whether the dominant phenotype is rescued by two wild-type gene copies (haplo-insufficiency) or not (dominant negative). The ready discovery of dominant negative (antimorphic) mutants in yeast and *Drosophila* suggest that this is a common property of actin and likely to be represented among *ACTA1* mutants. In F-actin, each monomer contacts four neighbours, so monomers mutated at the site of one interaction may still incorporate into the growing filament, but inhibit further polymerisation—creating 'dead ends" [38].

Overall it is apparent that hemizygosity for *ACTA1* null mutants is associated with recessive inheritance, missense mutations largely with dominant disease. This is similar to NEM-causing mutations in other sarcomeric proteins: slow α-tropomyosin (*TPM3*) [16,17] and slow troponin T (*TNNT1*) [20]. Nonsense and frameshift mutations in *NEB* are also associated with recessive NEM, but do not appear in most cases to be null alleles. Rather, alternative splicing around the mutations produces shortened nebulin polypeptides [19,39], similar to the reduced-size dystrophin in Becker muscular dystrophy [40]. We discuss below how the *ACTA1*missense mutations Leu94Pro and Glu259Val may cause recessive disease, whereas the other missense mutations cause dominant disease. Missense mutations in other actins, notably in yeast actin, can cause either dominant or recessive phenotypes [29].

3.3. Do actin mutants causing skeletal muscle disease cluster within the actin structure?

If the dominance of most *ACTA1* disease mutants is caused by disruption of specific actin interactions within the sarcomere, one might expect the mutant residues to cluster on the surface, or just below it, especially within or close to the binding sites for actin-binding proteins (ABPs). Important interactions would include actin–actin contacts in F-actin, contacts with other sarcomeric proteins, or with proteins involved in actin filament assembly, such as profilin, gelsolin, etc. *ACTA1* mutants (Table 2, Fig. 2) mapped onto the actin atomic structure (Fig. 4) show no obvious clustering, consistent with statistical analyses, which demonstrate an absence of mutant clustering in the protein sequence, in the subdomains or within the 3-D structure (Sparrow, unpublished). The absence of mutants clustering within actin may be due to a number of reasons, none of them mutually exclusive.

First, actin binds to a large and diverse array of ABPs in eukaryotic cells. Even in muscle myofibrils one can readily identify a number of such proteins, including myosin heavy chain and essential light chain, tropomyosin, troponin I (possibly troponin T), α-actinin, nebulin, titin, tropomodulin and CapZ and a number of enzymes with metabolic functions [41]. The transient binding of other ABPs such as profilin, thymosin β4 and cofilin/ADF, may be important for thin filament assembly during myofibrillogenesis. Few actin binding sites have been accurately determined, but when one includes the actin–actin contacts and the sites for the above ABPs, there may be few areas of the actin surface not involved. While defects in actin–actin contact sites will affect polymerisation, other mutations outside these sites may affect actin conformation and thereby polymerisation. A consequence of the number of actin–actin and actin–ABP interactions is that many different mutants are likely to disrupt actin binding and lead to aberrant sarcomeric assembly and function.

Second, the *ACTA1* myopathies are not a homogeneous group. By clinical and histological criteria it is currently believed that these myopathies can be assigned to a minimum of three classes—AM, IRM and NEM (Table 1). *ACTA1* mutations causing these different myopathies may cluster within actin, reflecting specific molecular dysfunctions.

3.4. Which actin functions are affected by the disease-causing mutations?

Our purpose in what follows is to use the knowledge of actin structure and function gained from model systems to attempt a prediction of the molecular consequences of specific *ACTA1*mutations. We hope to provide insights into how the disease phenotypes arise, or at least to detect

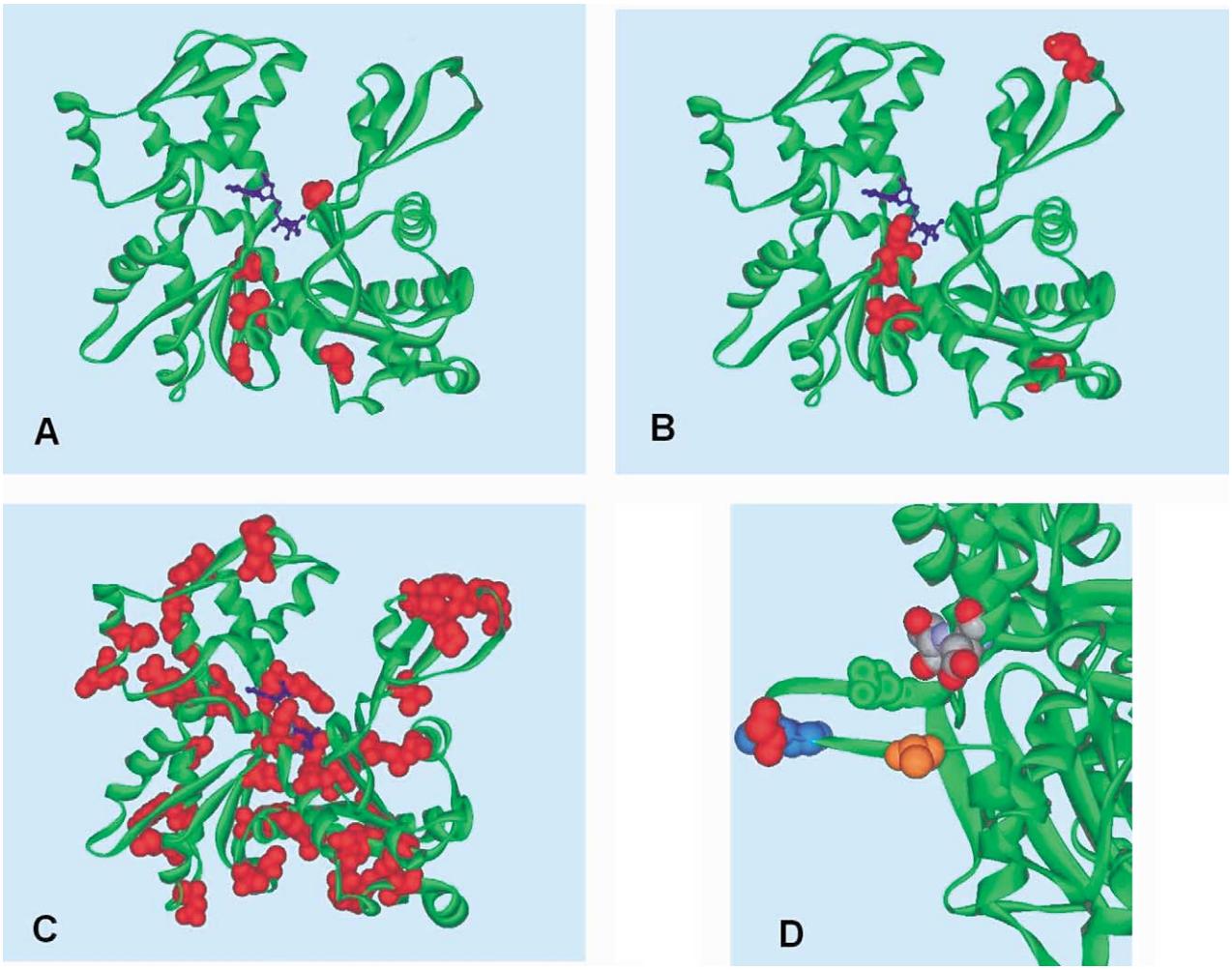

Fig. 4. Location of *ACTA1* mutations within the actin structure. Actin is represented by the green ribbon; ATP in blue. (A) AM mutant residues shown as atomic structures in red; (B) IRM mutant residues; (C) NEM mutant residues. Structures produced as in Fig. 3. (D) Detail of the actin atomic structure to show the 'plug' and residues referred to in discussing NEM mutants which may affect its functions. Gly268 is coloured red, Met269 in blue, Glu263 in light green and Ala272 in orange (these correspond to NEM plug mutations). Thr260 and Glu224 are shown in CPK colours. Glu224 is also the site of an NEM mutation. Panel D was produced using an atomic coordinate data set developed by Holmes et al. (1990) to model F-actin from the G-actin coordinates. JCS thanks Prof K.C. Holmes for providing this dataset.

patterns, propose testable models and perhaps indicate other candidate proteins for AM, IRM and NEM.

Molecular modelling of all the known *ACTA1* mutations is beyond the scope of this review and would not often predict, with any certainty, the functional effects of the mutant protein. So we have examined the position and likely direct role of the actin residues involved in *ACTA1* mutations and projected the immediate effects of the amino acid substitutions. All of this must be regarded as speculative as the functional effects in vivo are difficult to predict.

Atomic structures of actin have been obtained from co-crystals of G-actin with actin-monomer binding proteins, including DNAse 1 [42] gelsolin fragment 1 [43] and profilin [44]. Recently a structure was obtained from crystals of G-actin [45]. Overall, the structures are similar, though with local differences especially in regions to which the monomeric ABPs are bound, or depending on the nucleotide state. A model of F-actin structure has also been obtained [34] and further refined [46].

3.4.1. Actin myopathy (AM)

ACTA1 mutations causing AM are Gly15Arg, Gly146Asp, Asp154Asn, Val163Leu, Ser348Leu and a duplication of Ala144/Ser145. All the missense mutations are in very highly conserved residues [29], suggesting that the AM mutations are confined to residues crucial for actin function.

All the AM-causing *ACTA1* mutants are in subdomains 1 and 3, close to the central hinge at the base of the cleft and the nucleotide-binding pocket.

(i) Gly15 is part of a loop that binds ATP. Replacement of glycine by the much larger, charged arginine is likely to affect nucleotide binding. The neighbouring yeast actin mutation Ser14Ala resulted in significantly reduced ATP affinity and had structural effects on F-actin [47].

(ii) Gly146 is close to the 'hinge' and the base of a loop involved in ATP binding. It is likely indirectly to affect nucleotide exchange.

(iii) The Asp154Asn substitution will reduce charge interactions with the magnesium ion and affect nucleotide binding. The divalent cation is crucial for nucleotide binding and is located in a pocket formed by the nucleotide and residues Asp11, Asp154, Lys18 and Gln137.

(iv) Modelling of the Val163Leu and Ala138Val mutations (Clayton & Sparrow, unpublished), the latter, a severe dominant negative actin mutant in the *Act88F* flight muscle actin gene of *Drosophila* [27], shows spatial conflicts in incorporating the new mass resolvable only by distorting the hinge region. The hinge is crucial to opening of the actin cleft, which in turn is important for nucleotide exchange. Intriguingly the *ACTA1* IRM and NEM mutant, Ala138Pro will disrupt the hinge region severely.

(v) Ser348Leu occurs in a surface α-helix, a continuation of an α-helix which forms the hinge, and will cause a packing conflict with residues Ala22 and Gly23. Whether this changes hinge flexibility or indirectly affects ATP/divalent cation binding through distortion around Ala 22 and Gly23 remains unclear.

(vi) The final AM group mutation is a duplication of two residues, Ala144 + Ser145, which will lengthen the polypeptide chain and likely (indirectly) affect the actin hinge and actin flexibility or possibly change the position of the subdomain 3 'P-loop' and ATP binding.

All of the AM mutants therefore seem likely to affect nucleotide binding and exchange.

3.4.2. Intranuclear rod myopathy (IRM)

The actin mutants that cause IRM are His40Tyr, Ala138Pro, Asp154Asn, Val163Leu, Val163Met, Lys336Ile and Ile357Leu. These mutants do not cluster and clearly do not define a single binding site.

(i–iii) Val163Leu and Ala138Pro described above, along with Val163Met are likely to affect the hinge, with effects on cleft opening and nucleotide exchange.

(iv) Asp154Asn (see above) is part of a phosphate-binding loop and will affect nucleotide binding.

(v) Lys336 lies within the 'hinge' and forms part of the nucleotide-binding pocket. Clearly all these residues, as with those causing the AM phenotype, are likely to affect nucleotide binding or exchange.

(vi, vii) Neither His40 nor Ile357 are close to the hinge or the nucleotide-binding site, but a major actin–actin interaction along the F-actin long pitch helix involves residues 41–45 of subdomain 2 in one monomer and residue 375 of subdomain 1 in the monomer above. Chemical modification of His40 prevents polymerisation [48], suggesting that His40Tyr may have a similar effect. Residue 357 lies close to residue 375 which was not present in the G-actin atomic structure [42] used to make the F-actin model [34]. So how close Ile357 is to this contact is difficult to judge. Residue 357 was not included in the actin–actin contact sites [34,49]. His40Tyr and Ile357Leu are not close to the other mutations in the IRM group which cluster around the hinge. However, Orlova and Egelman [50] propose that subdomain 2 rotation occurs within F-actin following ATP hydrolysis and phosphate release. The functional consequences of this movement are not clear, though it affects F-actin flexibility [50] and interactions with myosin [51,52,53]. Evidence for communication between the nucleotide pocket, subdomain 2 and the C-terminus is now considerable [50,54,55,56,57,58] and suggests how the IRM-causing *ACTA1* mutations may be functionally linked to nucleotide binding.

The presence of nuclear F-actin is well established, but why and how specific *ACTA1*mutations lead to intranuclear rods remains a puzzle.

3.4.3. Nemaline rod myopathy (NEM)

NEM is the commonest *ACTA1*mutant phenotype. The NEM mutant residues are not clustered (Fig. 4), nor do they tend to occur on the surface where ABP-binding sites are

likely. This makes it difficult to discern functional patterns among the mutant changes.

In the following section, we have grouped the mutant residues by their likely effects. It is very important to remember that these effects are functionally interrelated. Thus changes in G-actin conformation and actin–actin contacts will affect polymerisation and the stability, functions and conformations of F-actin. Similarly, nucleotide exchange will be affected by hinge flexibility and interactions that stabilise cleft closure. Most actin functions are intimately related to the binding and hydrolysis of nucleotide.

(i) A single NEM *ACTA1* mutation, Gly182Asp, will affect nucleotide binding directly. Gly182 is part of the nucleotide pocket and the Asp substitution will disturb nucleotide binding and the position of Arg183 which hydrogen bonds to Ser14 and Glu71 as part of cleft closure (see below).

(ii) Interactions between residues either side of the cleft are important for its closure. The NEM mutations His73Leu/Arg, and Asp179Asn/His will affect the major stabilising His73 to Asp179 interaction, as genetic and biochemical studies of yeast actin have shown. Arg183 hydrogen bonds to Ser14 and Glu71 and substitution by smaller, uncharged residues, Arg183Cys/Gly/Ser, will affect cleft closure. Asn115 is a buried residue not involved in cross cleft bonds, but Asn115Ser and Asn115Thr may increase movement of the Pro70-Asn78 loop and thereby affect the His73–Asp179 interaction. Ile75Leu, with its reduction in residue size may produce similar effects.

(iii) Three NEM mutations, Ile136Met, Ala138Pro and Leu140Pro will disrupt the hinge, the latter two most severely.

(iv) Extensive inter-molecular contacts along the long-pitch F-actin helix involve residues 322–325 with 243–245; 286–289 with 202–204; and 166–169 and 375 with 41–45 [34]. Further interactions occur across the helix between residues 110–112 on one monomer and 195–197 on the opposite one. NEM mutations likely to affect these contacts include Gln41Arg, Gly42Val, Val43Phe, Glu241Lys and Asp286Gly. Asp286 is involved in a salt bridge with Arg39 and Glu270, all on different monomers [34], that will not occur when glycine is substituted. Proline residues restrict polypeptide chain flexibility and Pro38Leu is likely to severely affect subdomain 2 structure and movement. Some mutant substitutions close to actin–actin contacts could affect them, including Arg256His/Leu and Ile64Asn. In the G-actin structure of Otterbein et al. [45] Ile64 lies close to these residues. Introducing a large residue capable of making charge interactions, may affect actin binding through residues 41–45. Interestingly, Ile64Asn and Gln41Arg may disrupt the hydrophobic interactions important in the docking of the proposed cross-strand 'plug' (see below and Fig. 4). The Gln246Arg and Gln246Lys mutations introduce charge changes close to an actin binding site (residues 243–245).

(v) Holmes et al. [34] proposed that a 'plug' from one actin monomer occupies a 'pocket' formed between two monomers in the opposing F-actin strand. Residues Phe266–Met269 form the apex of the plug in an actin monomer on one strand, while the 'pocket' is formed by residues Tyr166, Ala169, Leu171, Cys285 and Ile289 in one monomer and residues Gly63, Ile64 and 40-45 of another in the opposite strand. The plug and pocket structure is not seen in the G-actin atomic structures [42,43,44,45]. However, yeast mutants in this loop affect actin polymerisation and F-actin stability [59,60,61,62] consistent with the plug being important in inter-strand links. Three *ACTA1* NEM mutations occur in the plug itself, Gly268Arg/Cys and Met269Arg and will affect its docking into the hydrophobic pocket (Fig. 4). Gly268Arg causes a more severe phenotype than Gly268Cys. This correlates with the problem of inserting a large charged side-chain (Arg) rather than a smaller, uncharged one (Cys) into a hydrophobic environment. However, both substitutions will also reduce the flexibility associated with the glycine at the plug apex. The correlation breaks down with Met269Arg which produces a mild NEM phenotype. Gln263Leu and Ala272Glu are in opposing strands in the 'stem' when the plug is modelled in its extended form [34]. When the loop is not extended [42,45] Gln263 can hydrogen bond with Glu224, which cannot occur in the Gln263Leu mutant. Interestingly Glu224Gln and Glu224Gly may affect this same interaction. In summary, Gln263Leu and Ala272Glu probably affect the docking of the loop against the surface, perhaps with consequences for deployment of the plug during polymerisation. A polymerisation-dependent movement of the loop has been detected in yeast actin mutants [63].

(vi) Val35Leu, Met132Val, Val134Ala and Glu259Val are NEM mutants in completely buried residues and probably affect the internal packing of actin. Leu94 is also completely buried, lying in an α-helix between Glu93 and Asp95, part of the proposed myosin secondary binding site. Insertion of proline, Leu94Pro, will disrupt the helix and probably much more. Glu259Val and Leu94Pro are the only recessive missense mutations. The other recessive NEM mutations, are one nonsense, one frameshift and one splice-site mutation. Perhaps the effects of these two missense mutations are so severe that their products lack sufficient structure to interfere with wild-type actin in heterozygotes.

(vii) Some *ACTA1* NEM mutations, Thr77Ala, Thr148Asn, Ala170Gly, Arg196Leu, Glu224Gln/Gly, Met227Ile/Thr/Val, Gly251Asp, Arg256His/Leu, Tyr279-His, Asn280Lys and Met283Lys are at, or close to, the actin surface, without readily predictable effects on actin structure or actin–actin interactions, though in some cases it is clear that stabilising interactions will be compromised, e.g. Tyr279His will lose hydrophobic interactions with Leu320 and Met283. Such mutations are likely to interfere with actin–ABP interactions.

(viii) The effects of the C-terminal mutants, Val370Phe, Arg372Ser, Lys373Gln, are difficult to predict as residues 373-5 are not seen in atomic structures.

In summary, the *ACTA1* NEM mutations are not clustered and will affect several primary actin functions.

3.4.4. Potential actin–ABP interactions affected by ACTA1 myopathies

Interaction sites of ABPs have been deduced by 'docking' binding partners and actin atomic structures, restricted by the 'mass envelopes' determined by image processing of high resolution EMs. This approach is not accurate enough to assign confidently particular residues to many of the contacts and largely ignores conformational adjustments involved in protein binding. However, through a combination of biochemical and genetic studies [64–66], residues have been assigned to binding sites.

3.4.4.1. Actomyosin. The actin residues involved in actomyosin interactions were predicted [67] by inserting the chicken myosin S1 atomic structure [68] and an F-actin model [34], into image reconstructions from EMs of S1-decorated F-actin. The myosin head apparently binds to two neighbouring actin monomers. Three NEM mutations, Asp25Asn, His40Tyr, Gln41Arg and Gly42Val occur in the predicted primary myosin binding site; Leu94Pro in the secondary binding site will severely disrupt local folding (see above). Ser348Leu (AM) lies in the myosin binding site though whether this conservative substitution will affect this interaction is unclear.

3.4.4.2. Actin-tropomyosin. Tropomyosin (Tpm), is a dimer, which by end-to-end association forms a continuous filament wound around F-actin. It has an important role in the control of striated muscle contraction (see above) but also probably stabilises F-actin in vivo. F-actin decorated with Tpm in the presence/absence of Ca^{2+} and the myosin S1 fragment showed structures [69] representing the three thin filament regulatory states proposed by McKillop et al. [70]. Gordon et al. [31] projected the Tpm in these reconstructions onto the actin surface to determine the actin residues below Tpm in each position. Some *ACTA1* mutations occur in residues able to interact with Tpm including Gln41Arg (NEM), the Ala144/Ser145 duplication (AM), Gly146Asp (IRM), and Thr148Asn (NEM). The increased polypeptide length of the duplication, or increased side-chain length, Gly146Asp, Thr148Asn, seem likely to protrude from the actin surface and affect Tpm movement. No other explanations for the effects of Thr148Asn seem more likely. For the other mutations, alternative explanations have been offered (see above).

3.4.4.3. α-Actinin. α-Actinin is an F-actin cross-linker and a major Z-disc component. EM studies of F-actin decorated with an N-terminal α-actinin domain [71] showed binding to two neighbouring actin monomers, consistent with biochemical studies that localised binding to residues 86–117 and 350–375 [72,73,74,75]. *ACTA1*NEM mutations possibly affecting α-actinin binding include Ala114Thr, Asn115Ser/Thr, Arg116His, Ile357Leu, Val370Phe, Arg372Ser and Lys373Gln. The resolution of the EM approach prevents determination of the C-terminal α-actinin binding site and whether the Ile357Leu, Val370Phe, Arg372Ser and Lys373Gln changes are likely to affect α-actinin binding.

3.4.4.4. Nebulin. Nebulin is a giant filamentous protein, spanning the thin filament length. It may act as a template to control thin filament length during myofibrillogenesis [76]. EM reconstructions of F-actin decorated with a nebulin fragment [77] show unexpectedly that nebulin binding occurs at three separate locations, suggesting that nebulin lies on, or moves between, different positions on the F-actin helix. *ACTA1* mutations in the nebulin contact sites include Met227Ile/Thr/Val, Arg256His/Leu, Glu259Val and Ile357Leu. This is the first functional assignment for residues Met227 and Arg256 and with Glu224 they may form an *ACTA1* NEM mutational cluster.

4. Discussion

ACTA1 mutations cause three histological types of myopathy, AM, IRM and NEM. If these are distinct myopathies, then the mutant effects on specific actin functions might be restricted to one myopathy. We predict that all the AM mutations will affect nucleotide binding and/or hinge flexibility. Similarly most of the IRM mutations seem likely to affect nucleotide binding and hinge flexibility while the remainder can be included by considering evidence of communication between events at the nucleotide pocket, the hinge and subdomain-2 movement. Our analysis suggests that AM and IRM mutations affect similar aspects of actin function, yet histologically the phenotypes, especially the nuclear rods, seem very distinct. Why this should be, is unclear. Only one NEM *ACTA1*mutation is predicted to affect nucleotide binding or actin flexibility. The remainder seem likely to affect G-actin conformation, or F-actin stability, either through actin–actin contacts, including the 'plug', or through interactions with other proteins, e.g. tropomyosin and nebulin. This may explain why the residues affected in *ACTA1* nemaline myopathies fail to cluster in the actin monomer. In this group there is a substantial minority of mutations for which we can, as yet, offer no clear molecular predictions.

The observation that the R39X nonsense mutation, which is likely a null mutation, is recessive, leads us to suggest that hemizygosity for the *ACTA1* gene is not disease-causing. Similarly, *ACTA1* mutations that produce non-functional actin will have a recessive phenotype, such as the A144 frameshift mutation and probably Glu259Val and Leu94Pro. Conversely, the majority of *ACTA1* mutations

are dominant and must produce their phenotypes by dominant negative effects. However, since they produce three distinct histological phenotypes, it further suggests that their protein products interfere with different aspects of actin function. Almost certainly, the questions we should be asking from our predictions are not what actin functions are altered in the mutant proteins, but how the functions they retain interfere with the ability of the wild type proteins to function normally. For instance, if a mutant actin has altered nucleotide binding/hinge flexibility, what are the consequences on the pools of wild type G-actin monomers and their ability to bind to the growing end of an F-actin filament? Do mutant actin monomers compete with wild-type monomers for the growing end of the F-actin filaments and prevent/slow the whole assembly of F-actin by preventing the binding of further wild-type monomers in the 'dead-end' hypothesis we proposed some years ago [38]?

All the nemaline myopathies characterised to date involve mutations of thin filament proteins—nebulin, actin, tropomyosin and troponin T. Our analysis of *ACTA1* NEM mutations suggests that most affect G- and F-actin polymerisation, F-actin stability and interactions with tropomyosin or nebulin, while there are no mutations in the quite large myosin binding site except where other explanations are possible and only a minority of the *ACTA1* NEM mutations can have a primary effect on actin α-actinin interaction. This latter indicates that any hypothesis that nemaline bodies form through impaired actin α-actinin interactions in the Z-disc is simplistic. Tropomyosin enhances actin polymerisation and is believed to stabilise F-actin in vivo. Nebulin seems likely to do the same. How does a troponin-T mutation also produce a nemaline myopathy? There is no evidence that troponin-T contacts actin. A more likely explanation is that troponin T, binding tropomyosin at the overlap region between successive tropomyosin dimers, stabilises the tropomyosin and indirectly affects the F-actin core of the thin filament. The only troponin T nemaline mutation identified to date is a nonsense mutation at residue 180 that causes recessive nemaline myopathy in the Amish [20]. Absence of troponin T likely destabilises the thin filament. Conversely if *ACTA1* mutations destabilise thin filaments, then one would have predicted mutations with similar effects in just these three other thin filament proteins. Other candidate proteins for NEM and the other diseases are not obvious.

Many questions remain about the ontogeny of these skeletal myopathies and they are difficult to study in patients. For example, exactly how nemaline bodies form remains unknown and will probably remain so until reproducible, reliable tissue culture models are devised. One intriguing feature of the myopathies is why the effects on sarcomere structure, often extreme, are localised next to normal areas within the muscle fibres. Maybe it is due to a 'catastrophe' effect that leads to local destabilisation/absence of assembly? Another important question is whether the sarcomeric effects are direct developmental effects or represent degradative conditions or both.

More information on the actin functions affected by the mutations, or, more accurately, what the effects of the mutant actins are on wild type actin function, is required to gain insight into the molecular effects of the *ACTA1* mutations. Most of the predictions above could be tested biochemically on expressed mutant actins in vitro, but actin expression is difficult [29]. Additionally we need in vivo models to understand the ontogeny of these myopathies. Intriguingly, mutations are already known in the *Drosophila Act88F* gene at four residues where *ACTA1* NEM mutations occur. Flies heterozygous for these mutations produce structures with close homology to nemaline rods (Haigh, Nongthomba and Sparrow, unpublished). The use of this model, transgenic mice (a transgenic mouse model of *TPM3* NEM has been generated [78]) and in vitro muscle development, should provide insights into how the *ACTA1* mutations subvert normal muscle development and maintenance.

Understanding the pathobiology of the *ACTA1* mutations is essential for rationally devising treatments of the actin diseases and those caused by mutations in other sarcomeric proteins [79], though empirical testing may be successful.

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