

Nemaline myopathies

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ACTA1	skeletal muscle α -actin gene
CCD	central core disease
NEB	nebulin gene
RYR1	ryanodine receptor gene
TNNT1	slow troponin T gene
TPM2	β -tropomyosin gene
TPM3	slow α -tropomyosin gene

Definition of entities

Nemaline myopathies are now considered as a genetically diverse group of congenital myopathies marked by excessive formation of rods or nemaline bodies within muscle fibres and/or, more rarely, nuclei.

Shy et al (23) as well as Conen et al (2) are usually credited with having first described nemaline myopathy. The latter group named the nemaline bodies “myogranules,” a non-specific term, which did not earn full acceptance by the scientific community. However, it is now clear that in 1958 the Australian pathologist Douglas Reye discovered “nemaline bodies” in the muscle biopsy of a boy, who some 40 years later was found to have a mutation in the ACTA1 gene (22).

Four broad clinical types of nemaline myopathy have been described (15, 28): a severe neonatal form, a much milder congenital or “classic” form, a late or adult onset form—all following an autosomal recessive mode of inheritance—and a separate autosomal dominant form commencing in childhood.

Clinically, the nemaline myopathies present with proximal or generalized muscle weakness sparing bulbar muscle innervated by cranial nerves except facial muscles, which may be severely affected. This results in a characteristic long face, a high arched palate, and a tent shaped mouth. Respiratory insufficiency may remain a threat in early onset forms and may be a major feature in the adult form. Occasional arthrogyposis may reflect intrauterine onset

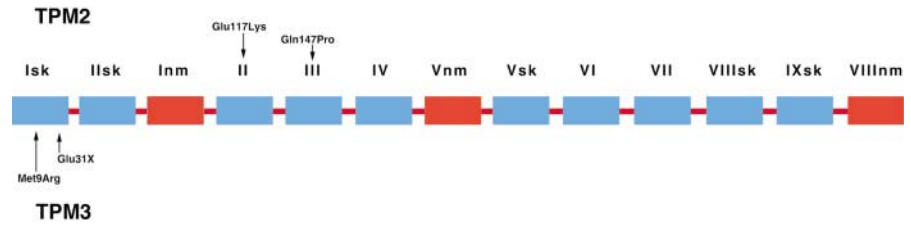


Figure 1. Genomic structure of the tropomyosin genes. Mutations in slow- α tropomyosin (TPM3) and β tropomyosin (TPM2) associated with nemaline myopathy. Red-stained exons are alternatively spliced exons in the non-muscle isoforms of the protein.

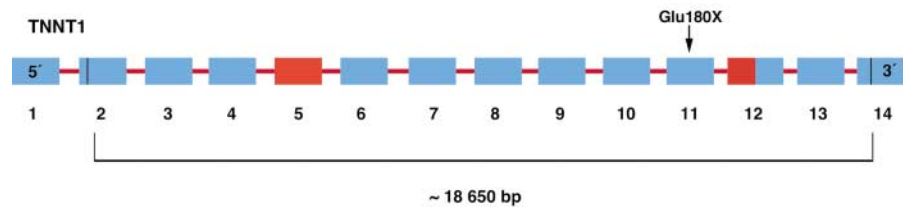
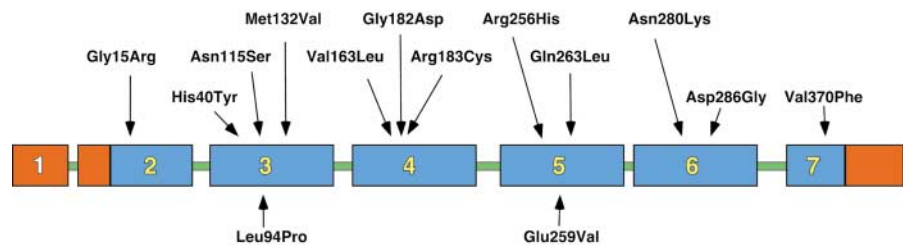


Figure 2. Genomic structure of the slow troponin T gene (*TNNT1*). Position of the nonsense mutation identified in the Amish population. Red-stained exons are alternatively spliced exons.

Dominant



Recessive

Figure 3. Genomic structure of the skeletal muscle α -actin gene *ACTA1* and the published mutations in *ACTA1* associated with muscle disease.

(4, 21). The presence of intranuclear rods may indicate a serious prognosis (1, 6, 19). The heart may occasionally be involved morphologically both in children (24, 27) and adults (13, 25).

Molecular genetics and pathogenesis

The first 2 nemaline myopathy disease genes to be identified, slow α -tropomyosin (*TPM3*) and nebulin (*NEB*) were discovered through positional cloning (12, 18). Both of the encoded proteins are components of the

thin filament, and subsequent candidate gene approaches or positional candidate approaches have been directed at other thin filament proteins. This has resulted in the identification of mutations associated with nemaline myopathy in three other thin filament protein genes—skeletal muscle α -actin (*ACTA1*) (17), β -tropomyosin (*TPM2*) (3) and slow troponin T (*TNNT1*) in the unique autosomal recessive nemaline myopathy seen in the Amish community (10).

In addition, mutations in the ryanodine receptor gene (*RYR1*), more usually associated with either malignant hyperthermia or central core disease (CCD), lead to the production of nemaline bodies (14, 20). Thus, while mutations of five thin filament proteins and one sarcoplasmic reticulum protein have been associated with the formation of nemaline bodies, the major proteins of Z bands and rods, α -actinin 2 and α -actinin 3, have not yet been found as a mutant form in nemaline myopathy. In fact, absence of α -actinin 3 is not associated with disease (16).

Mutations have been identified in *ACTA1* and *TPM3* which lead to both dominant and recessive diseases, while mutations in *NEB* and *TNNT1* have to date been associated with recessive conditions (Figures 1-4).

The dominant mutation first identified in *TPM3*, was a Met9Arg missense mutation (12). The dominant mutations in *TPM2* are missense mutations (3) (Figure 1) and the vast majority of the missense mutations in *ACTA1* are dominant mutations (17) (Figure 3). The published recessive mutation in *TPM3* is a nonsense mutation (26) (Figure 1), as is the recessive mutation identified in *TNNT1* in the Amish nemaline myopathy (10) (Figure 2). In contrast, most of the recessive mutations in *NEB* are frameshift mutations (18) (Figure 4). Thus, there is a pattern emerging in the thin filament diseases of missense mutations being associated in the main with dominant disease while nonsense, or null mutations, are associated with recessive disease.

To date, close to 50 almost exclusively missense mutations in the *ACTA1* gene have been identified (Laing N, unpublished observations). Most frequently, the patients with *ACTA1* mutations are isolated cases with no previous family history, though the disease is inherited in a dominant fashion in some of the milder forms. In every isolated case examined to date where it has been possible to examine DNA from the parents, the *ACTA1* mutation has been shown to be a de novo mutation. This indicates a very

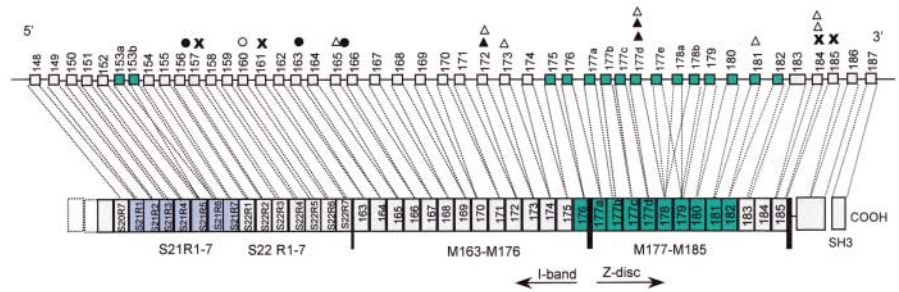


Figure 4. Mutations in the 3' end of the nebulin gene from exon 148 to exon 187 corresponding to the C-terminal end of the protein, from repeat 7 in superrepeat 29 (S20R7) to the SH3 domain. The exons are shown as boxes numbered from 148 to 187, and the introns are shown as lines, above the protein structure. Alternatively spliced exons are shown as grey boxes. Mutations are indicated by symbols, one for every mutation identified: X, nonsense mutation; open circles, missense mutations; closed circles, splice site mutations; open triangles, small deletions; closed triangles, small insertion. By courtesy of Dr L. Pelin and Dr C. Wallgren-Pettersson, Helsinki, Finland.

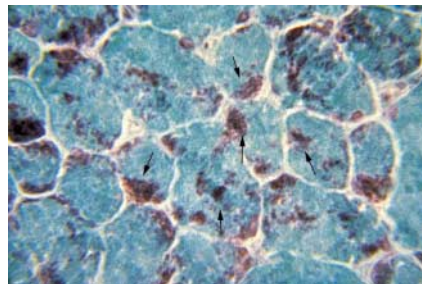


Figure 5. Numerous stained rods within muscle fibres. Examples indicated by arrows. Modified trichrome stain.

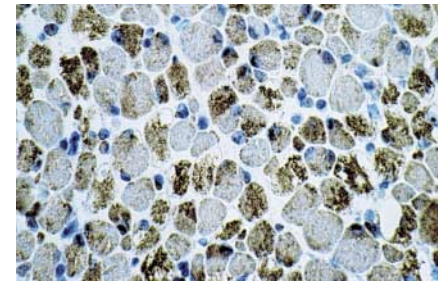


Figure 6. Rods marked by antibody against α -actinin, the chief protein of the Z disk. Immunohistochemistry.



Figure 7. By electron microscopy, rods are situated close to the nucleus under the sarcolemma (arrows) or deeper inside the muscle fibre.



Figure 8. Rods appearing at higher magnification with irregular size and intrinsic structure.

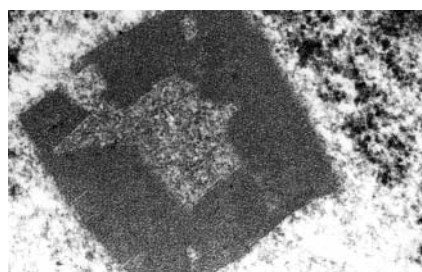


Figure 9. Intranuclear rods. By electron microscopy the intranuclear rod displays the characteristic criss cross pattern of Z disks.

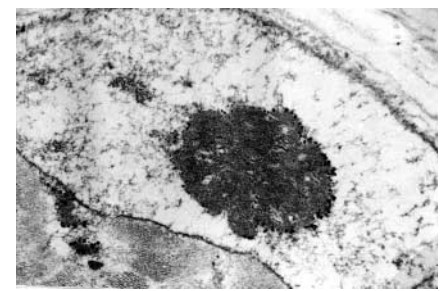


Figure 10. By immunoelectron microscopy, the antibody against α -actinin, the chief protein of the Z disk, labels the intranuclear rod, and the Z disks close to the nucleus as an internal control. Immunogold technique.

high rate of spontaneous new mutations (11). Mutations in the α - and β -tropomyosin genes have been found in only a few families, suggesting that such mutations represent only a minor component within the genetic spectrum of nemaline myopathies whereas mutations in the nebulin gene on chromosome 2q21.2-22 are more numerous (18).

Structural changes

The formation of rods (Figures 5-10) within muscle fibres is the hallmark of nemaline myopathies. These rods often form clusters beneath the sarcolemma and they may occur as large bodies among myofibrils; their long axes often paralleling the long axes of the sarcomeres. Their origin from pre-existing Z bands can occasionally be seen.

The nemaline bodies represent a surplus of Z-band material predominantly composed of α -actinin, the major component of the Z-disc (Figure 6). The pathogenesis of the nemaline bodies is still unclear. The role of the mutant proteins—actin, nebulin, the tropomyosins, troponin T—and the ryanodine receptor in inducing, relating to rod formation, remains to be clarified.

Mutations in the *ACTA1* gene are linked to 2 peculiar morphological phenomena in muscle fibres: *i*) aggregation of actin filaments (5), thus forming an actinopathy and *ii*) the presence of rods within nuclei of muscle fibres: intranuclear rod myopathy. The pathogenesis of these changes is also uncertain. In both adults and in children affected by nemaline myopathy, rods have been seen within nuclei (Figure 9, 10) (7) and these rods usually, though not always, occur singly and have a rather large size. Whether they originate from soluble sarcoplasmic proteins which have entered the nucleus, or merely constitute invaginations and nuclear membrane penetration is still undecided, especially when rods may almost exclusively occur within nuclei (6).

By immunohistochemistry and immunoelectron microscopy, sarcoplasmic

and nuclear rods contain α -actinin (Figure 10) and sarcomeric actin, but nebulin, a large sarcomeric protein, has not been found abnormal (8). The formation of rod bodies, which are structured similarly to Z bands, and the presence of both α -actinin and α -actin within both the intranuclear and sarcoplasmic rods, indicate another surplus protein myopathy. However, this surplus protein myopathy is different from other forms. In actinopathy and in desmin-related myopathies containing the mutant proteins, ie, actin and desmin, surplus protein myopathy occurs in a filamentous arrangement, not unlike the Z-band-like structures of the rods.

Trains of rods may occasionally occupy circumscribed areas resembling and, perhaps, even forming cores. Such “rods in cores” now seem to be evidence of CCD (14, 20), rather than that of nemaline myopathies because mutations in the CCD specific gene locus *RYR 1* have been identified in such patients.

Cardiac myocytes may occasionally show rod-like abnormalities (9, 13, 25, 27), and these lesions often show electron dense material within cardiac sarcomeres rather than structured rods. Additional features within muscle biopsy specimens are type I fibre hypotrophy, often together with type II fibre predominance, and fulfilling the criteria of fibre type disproportion, the latter phenomenon also occurs in other congenital myopathies.

Future perspectives

Regular studies of families with nemaline myopathy have, surprisingly, opened a new view of this group of disorders documenting genetic heterogeneity which may even encompass additional long identified gene loci. To explain the pathogenetic role of mutant Z band-associated proteins in nemaline myopathies will be a major undertaking. Additional challenges will also present themselves; exploration of the complete composition of rods, both formed within the sarcoplasm as well as within nuclei, and explanation of intranuclear rod appearance.

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