Published in final edited form as:

Hum Mutat. 2010 February ; 31(2): 176–183. doi:10.1002/humu.21157.

Mutations of tropomyosin 3 (*TPM3*) are common and associated with type 1 myofiber hypotrophy in congenital fiber type disproportion

Michael W. Lawlor*, Elizabeth T. DeChene*, Emily Roumm, Amelia S. Geggel, Behzad Moghadaszadeh, and Alan H Beggs

Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research, Children's Hospital Boston, Harvard Medical School, Boston, MA

Abstract

Congenital fiber type disproportion (CFTD) is a rare congenital myopathy characterized by hypotonia and generalized muscle weakness. Pathologic diagnosis of CFTD is based on the presence of type 1 fiber hypotrophy of at least 12% in the absence of other notable pathological findings. Mutations of the *ACTA1* and *SEPN1* genes have been identified in a small percentage of CFTD cases. The muscle tropomyosin 3 gene, *TPM3*, is mutated in rare cases of nemaline myopathy that typically exhibit type 1 fiber hypotrophy with nemaline rods, and recently mutations in the *TPM3* gene were also found to cause CFTD. We screened the *TPM3* gene in patients with a clinical diagnosis of CFTD, nemaline myopathy, and with undefined congenital myopathies. Mutations in *TPM3* were identified in 6 out of 13 patients with CFTD, as well as in one case of nemaline myopathy. Review of muscle biopsies from patients with diagnoses of CFTD revealed that patients with a *TPM3* mutation all displayed marked disproportion of fiber size, without type 1 fiber predominance. Several mutation-negative cases exhibited other abnormalities, such as central nuclei and central cores. These results support the utility of the CFTD diagnosis in directing the course of genetic testing.

Keywords

Tropomyosin; *TPM3*; CFTD; fiber type disproportion; congenital myopathy; skeletal muscle; pathology; type 1 fiber; hypotrophy; atrophy

Introduction

Congenital fiber type disproportion (CFTD) (CFTD forms 1–2; MIM#s 255310, 300580) is a rare congenital myopathy characterized by hypotonia and generalized muscle weakness. The symptomatic severity of patients with CFTD is highly variable, ranging from severe weakness and death in infancy to extremely mild deficits seen only in adulthood (Clarke and North, 2003). Diagnosis of CFTD is based on the presence of type 1 fiber hypotrophy of at least 12% in the absence of other notable pathological findings, in addition to a clinical presentation typical of congenital myopathies. Some authors set a higher threshold for hypotrophy in CFTD, and prefer to make the diagnosis only in cases where type 1 fiber hypotrophy exceeds 25%. Type 1 fiber hypotrophy is a common finding in other neuromuscular conditions, making it essential to exclude other potential diagnoses before

Corresponding Author: Alan H. Beggs, Ph.D., Genetics Division, Enders 5, Children's Hospital Boston, 300 Longwood Ave, Boston, MA 02115, Tel: (617) 919-2170, Fax: (617) 730-0786, beggs@enders.tch.harvard.edu.

*These authors contributed equally to the work.

confirming CFTD. Not only are the clinical presentations highly variable among patients, but CFTD also exhibits genetic heterogeneity with X-linked, autosomal dominant, and autosomal recessive inheritance patterns (Clarke and North, 2003). To date, causative mutations have been identified in three genes, α -tropomyosin_{slow} (*TPM3*; MIM# 191030) (Clarke, et al., 2008), skeletal muscle α -actin (*ACTA1*; MIM# 102610) (Laing, et al., 2004) and selenoprotein N 1 (*SEPN1*; MIM# 606210) (Clarke, et al., 2006). While *ACTA1* and *SEPN1* mutations are rare causes of CFTD, a recent paper by Clarke et al. (Clarke, et al., 2008) identified six different *TPM3* mutations in CFTD families, four of which account for 25% of CFTD cases in a single Australian cohort.

Tropomyosins are actin-binding, coiled-coil proteins expressed in all eukaryotic cells. Their major function in skeletal muscle is to stabilize actin and to regulate actin/myosin interactions by limiting access to myosin binding sites along the major groove of the actin filament (Gunning, et al., 2005). Tropomyosins are produced by alternate splicing of at least 4 vertebrate genes, *TPM1-4*. The three major different tropomyosin gene products expressed in skeletal muscle are α TM_{fast} (Tropomyosin 1/*TPM1*), β TM (Tropomyosin 2/*TPM2*), and α TM_{slow} (Tropomyosin 3/*TPM3*; MIM# 191030) (Lees-Miller and Helfman, 1991). The *TPM3* gene, located at 1q22-23, encodes a muscle-specific isoform that is primarily expressed in slow/Type 1 skeletal fibers. Mutations in *TPM3* are a rare cause of nemaline myopathy (NM), a congenital myopathy characterized by nemaline bodies on muscle biopsy, and there is one report of *TPM3* mutation in a rare but possibly related myopathy called cap disease (Ohlsson, et al., 2009). Six *TPM3* mutations with autosomal dominant or recessive inheritance have been associated with NM to date (Figure 1A) (Durling, et al., 2002; Laing, et al., 1992; Laing, et al., 1995; Lehtokari, et al., 2008; Penisson-Besnier, et al., 2007; Tan, et al., 1999; Wattanasirichaigoon, et al., 2002). Interestingly, one of the prominent histopathological features of many of these cases is type 1 fiber hypotrophy (Durling, et al., 2002; Laing, et al., 1992; Laing, et al., 1995; Penisson-Besnier, et al., 2007; Ryan, et al., 2003; Tan, et al., 1999; Wattanasirichaigoon, et al., 2002). These cases are pathologically similar to the changes seen in CFTD, with the added presence of nemaline rods in some type 1 fibers. Due to the pathological similarity between these cases of nemaline myopathy and most cases of CFTD, we conducted a clinicopathological analysis of a group of CFTD cases and screened the *TPM3* gene. While our study was in progress, Clarke et al. reported on a similar study in which they discovered mutations of the *TPM3* gene in 6 of 23 patients with CFTD. Here, we confirm and extend these findings. In addition, given that *TPM1*, a known cardiomyopathy gene, is also expressed in skeletal muscle and mutations of *TPM2* are associated with congenital myopathy, we performed an initial screen of the *TPM1* gene in patients with congenital myopathy and muscular dystrophy.

Materials and Methods

Using genomic PCR and DNA sequencing with primers specific for the 10 exonic regions (Tan, et al., 1999), we screened the *TPM3* gene (NCBI accession number NM_152263.2) in 78 unrelated patients, including 13 patients with referring clinicopathological diagnoses of CFTD, 37 with an undefined congenital myopathy, and 18 with onset of nemaline myopathy at five years or older or unknown age of onset. Using primers as described by the Genomics of Cardiovascular Development, Adaptation, and Remodeling. NHLBI Program for Genomic Applications, Harvard Medical School. URL: http://genetics.med.harvard.edu/~seidman/cg3/genes/TPM1_exons.html, we used the same techniques to sequence the 10 exons of the *TPM1* gene (NCBI accession number NM_001018005.1) in 65 unrelated patients, including 33 patients with a referring clinicopathological diagnosis of nemaline myopathy, 21 with other congenital myopathies, and 11 with undefined muscular dystrophies or limb girdle muscular dystrophy. All these

initial “referring diagnoses” were provided by referring clinicians. All study subjects provided appropriate informed consent and were enrolled under the supervision of the Children’s Hospital Boston institutional review board. None of the patients had known mutations in previously identified genes. Western blot analysis for tropomyosins in the muscles of Patients 311-1, 313-1, and 343-1 was completed as previously described (Wattanasirichaigoon, et al., 2002) using a NuPAGE 4–12% Bis-Tris gel and both monoclonal anti-sarcomeric tropomyosin antibody clone CH1 (α -TPM CH1; Sigma-Aldrich, St. Louis, MO) and monoclonal anti-tropomyosin clone TM311 (α -TPM 311; Sigma-Aldrich, St. Louis, MO). All nucleotide numbering is based on the cDNA sequence with +1 corresponding to the A of ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen). All mutation and relevant clinical data have been deposited into the Leiden Open Variation Database for *TPM3* (http://www.dmd.nl/nmdb2/home.php?select_db=TPM3). The pathology reports were reviewed in 55 of 78 cases screened for *TPM3* mutation, and slides of 12 of the 13 CFTD patients and the one NM case with *TPM3* mutation were reviewed by MWL, including 5 of 6 patients with *TPM3* mutations. Electron microscopy was performed or reviewed in 4 of the 5 unrelated CFTD cases with *TPM3* mutations. During our slide review, a revised pathologic “study diagnosis” of CFTD was made on the basis of decreased type 1 fiber size (>12%) in comparison to the type 2 fibers within the muscle biopsy, in addition to a lack of other findings within the biopsy specimen (Table 1) (Clarke and North, 2003; Dubowitz V, 2007; Jaffe, et al., 1988). A predominance of type 1 fibers (>55% of fibers in a quadriceps biopsy) was not used as a necessary diagnostic criterion in our analysis. Light microscopic images were captured using a SPOT Insight 4 Meg FW Color Mosaic camera and SPOT 4.5.9.1 software (Diagnostic Instruments, Sterling Heights, MI). Fiber size determinations were performed using point-to-point measurements across the minor fiber diameter using this software.

Results

For review of skeletal muscle pathology, patients were initially grouped according to the referring diagnoses made during their previous clinical and pathological workup, based on a combination of clinical symptoms, laboratory tests, and muscle biopsy findings. Using this stratification method, 13 patients were included in the CFTD group, 37 patients were included in the “undefined congenital myopathy” group, and 18 patients were included in the nemaline myopathy group. Pathologic diagnoses rendered in the cases of “undefined congenital myopathy” included skeletal muscle with fibrosis, neuropathic features, mixed myopathic and neuropathic features, type 1 fiber predominance, type 2 fiber atrophy, or end-stage muscle. Our arbitrary inclusion criteria for patients with nemaline myopathy included those who had become symptomatic at over 5 years of age or at an unknown age.

Our pathological analysis allowed us to correlate muscle biopsy findings with the presence of mutations in *TPM3*. Five different *TPM3* mutations were identified in 6 unrelated patients, all of which displayed marked type 1 fiber hypotrophy on muscle biopsy. Mutations included four heterozygous missense changes and one homozygous mutation of the normal stop codon leading to production of an abnormally large protein (Table 2, Figure 1A). As with the previously reported case that was compound heterozygous for p.X286Ser and another mutation, this change is clearly autosomal recessive as the parents are clinically unaffected and were found to be heterozygous carriers of the mutation. Remarkably, five of the six mutations were found among the CFTD cohort, including one autosomal dominant, one autosomal recessive, and three *de novo* changes. It was not possible to predict the type of *TPM3* mutation on the basis of histological findings. Upon parental testing, one heterozygous mutation was also identified in the previously undiagnosed mother (126-2) of patient 126-1. One of the 18 cases of nemaline myopathy (Patient 343-1) had an apparent *de*

*nov*o mutation, and no mutations were identified among the 35 undefined myopathy cases. No *TPM1* mutations were identified in any of the patients screened.

All previously unreported mutations were ruled out in at least 140 unaffected control individuals, including over 80 originating from a matched ethnic group. The previously reported c.503G>A (p.Arg168His) mutation had already been tested in 100 control individuals (Durling, et al., 2002). A search of the NCBI Entrez SNP database (<http://www.ncbi.nlm.nih.gov/sites/entrez>, January, 2008) did not identify any of these alterations. In addition, parentage was confirmed in the 3 *de novo* mutations (913-1, 311-1 and 247-4) using twelve highly polymorphic STR markers. Parental samples for the third isolated case (311-1) were unavailable.

In order to further characterize the effects of the mutations on the protein, Western blot analysis was performed for tropomyosins in the muscles of Patients 311-1, 313-1, and 343-1. Patients 311-1 and 343-1 showed no difference from the control (Figure 1B). Patient 313-1, which had the autosomal recessive mutation c.857A>C (p.X286Ser), has the same pattern as reported previously (Wattanasirichaigoon, et al., 2002), with a novel band approximately 6kD larger, representing the additional 57 amino acids following the read-through Stop → Ser change.

The majority of patients (5 of 7) with *TPM3* mutations presented in infancy, with hypotonia and delayed motor milestones being the primary symptoms (Table 2). Two patients diagnosed in adulthood retrospectively reported presenting in childhood or adolescence with difficulty running and/or keeping up with peers, as well as cramping and/or pain with exercise. All patients had myopathic facies or facial weakness, but none were noted to have extraocular muscle weakness. All six patients over 18 months of age were ambulatory, except for the patient with homozygous c.857A>C. Degree of muscle involvement varied from no recognized weakness to severe muscle weakness. None of the six patients with heterozygous changes were reported to require full-time ventilation or a feeding tube, although two of these patients required nighttime ventilation at some point in their life. Several patients were reported to have high-arched palates, mild scoliosis, and/or mild joint contractures or tightness of the fingers, heel cords, hamstrings, and/or iliotibial bands. Deep tendon reflexes were generally decreased or absent. Results of electromyography and nerve conduction studies were normal, myopathic, or mixed neuropathic and myopathic.

In our CFTD cohort, pathologic features of patients with a diagnosis of CFTD and the *TPM3* mutations primarily consisted of type 1 fiber hypotrophy in the absence of other pathologic findings (Figure 2A, B, and C). Type 1 fiber predominance was observed in at least 6 of the 13 cases with referring diagnoses of CFTD (Table 1), but was not a prominent feature of the *TPM3* mutation-positive cases (mean 48% ± 6% type 1 fibers for the group). Review of the slides and reports of patients referred with a referring diagnosis of CFTD revealed that additional pathologic findings, not typical for CFTD, and not seen in any of the mutation-positive cases, were present in 7 of the 8 mutation-negative cases, including excessive central nucleation, central cores, selective type 2 fiber atrophy, fibrosis, neuropathic changes, mitochondrial aggregates, or red-rimmed vacuoles (Table 1). Thus, these cases do not meet our stricter pathological “study diagnosis” criteria as defined in the Materials and Methods.

Discussion

Pathological analysis of CFTD patients in our study supports the use of type 1 fiber hypotrophy as an isolated finding, as a predictor of mutations in *TPM3*. There was marked type 1 fiber hypotrophy in all CFTD and NM patients with *TPM3* mutation. Six of the 8

mutation-negative cases referred as CFTD showed type 1 fiber predominance, but type 1 fiber predominance was not a feature of the CFTD cases with *TPM3* mutations ($48\% \pm 6\%$ type 1 fibers). Additionally, muscle biopsies of 7 of the 8 mutation-negative cases displayed pathological abnormalities in addition to type 1 fiber hypotrophy, which technically precludes the diagnosis of CFTD for these cases. These findings support the use of strict pathological criteria in the diagnosis of CFTD, since patients with strictly-defined CFTD are likely to have *TPM3* mutations.

Marked fiber type disproportion (type 1 fibers $> 47\%$ smaller than type 2 fibers) was seen in the 4 reviewed biopsies from CFTD cases with *TPM3* mutation, as well in the nemaline myopathy case with *TPM3* mutation. Biopsied muscle from the mother of patient 126-1 (patient 126-2) had a lesser degree of hypotrophy (type 1 fibers 33% smaller than type 2 fibers) than was observed in the other *TPM3* mutants, but she also displayed only mild symptoms, including ptosis, myopathic facies and mild muscle weakness. In addition, biopsies from patients with *TPM3* mutation displayed hypotrophy of most, if not all, type 1 fibers in the specimen, with no evidence of atrophy/hypotrophy of type 2 fibers or of fiber type grouping. These findings are similar to those reported in 3 cases of CFTD due to *ACTA1* mutation (Laing, et al., 2004), but in contrast, the 3 reported cases of CFTD due to *SEPN1* mutation involve a less homogeneous degree of type 1 fiber hypotrophy than is seen with either mutation of *TPM3* or *ACTA1* mutation, with severe hypotrophy seen in only a minority of type 1 fibers (Clarke, et al., 2006). These findings raise the possibility that the presence of a homogenous and high degree of fiber type disproportion affecting type 1 fibers may be predictive of mutations in either *TPM3* or *ACTA1*. Type 1 fiber predominance has been a common feature in previous reports of patients with *TPM3* mutations with both CFTD and nemaline myopathy (Clarke, et al., 2008; Ilkovski, et al., 2008; Laing, et al., 1992; Penisson-Besnier, et al., 2007; Ryan, et al., 2003; Wattanasirichaigoon, et al., 2002)). In our study, type 1 fiber predominance was not found in cases of CFTD with *TPM3* mutation. The proportion of type 1 fibers within a muscle is dependent on numerous factors, including the muscle biopsied, which are difficult to control in retrospective studies of muscle biopsy tissue. However, it should be noted that a recent paper by Ilkovski et al. (Ilkovski, et al., 2008) detected high percentages of type 1 fibers in several muscles of a patient with nemaline myopathy and a Met9Arg mutation in *TPM3*. While previous reports suggest that *TPM3* mutation may promote type 1 fiber predominance, our data do not support the inclusion of type 1 fiber predominance as a necessary diagnostic criterion for CFTD.

Remarkably, our nemaline myopathy patient (343-1) with the c.502C>T (p.Arg168His) mutation had the same *TPM3* mutation as one of our CFTD patients (247-4). On biopsy at 59 years, type 1 fiber hypotrophy and numerous nemaline rods within type 1 fibers were seen in patient 343-1's biopsy (Figure 2D, E and F), while patient 247-4, per report, had only type 1 fiber hypotrophy and no nemaline rods. This mutation was previously reported in 2 families with nemaline myopathy and one family with diagnoses of both CFTD and NM in different family members (Clarke, et al., 2008; Durling, et al., 2002; Penisson-Besnier, et al., 2007). Muscle biopsies described in these reports noted hypotrophy of many type 1 fibers, with nemaline rods found within type 1 fibers by light and electron microscopy in all but one patient. Clarke et al. reported a father and daughter with the p.Arg168His mutation in which the daughter had rods in fewer than 2% of type 1 fibers and the father had no identifiable rods, resulting in a diagnosis of CFTD (Clarke, et al., 2008). The restriction of pathologic findings to type 1 fibers is a feature that these cases have in common with the findings in patient 343-1's muscle biopsy.

The p.X286Ser mutation found in our CFTD patient 313-1 has been previously reported in a 5 year old boy with nemaline myopathy (Wattanasirichaigoon, et al., 2002). Interestingly,

both cases have Hispanic/Mexican ancestry, suggesting that p.X286Ser could be a founder mutation in this population. While numerous nemaline rods and excessive variation in fiber size are seen in the reported case of nemaline myopathy upon review, there does not appear to be selective hypotrophy of type 1 fibers (Wattanasirichaigoon, et al., 2002). This patient was a compound heterozygote for p.X286Ser and a second mutation, and he had a previous biopsy at age 2 years that was inconclusive. The biopsy of patient 313-1 in infancy shows marked atrophy of most type 1 fibers and a lack of nemaline rods, verified by electron microscopy. These findings raise the possibility of subsequent development of nemaline rods in patients with *TPM3* mutations as patients age, although the variability in histological findings could be due to the difference in mutations. However, it should be noted that biopsies taken from two of our patients with *TPM3* mutations at 11 (Pt. 247-4) and 29 (126-2) years of age, as well as a biopsy of a *TPM3* patient at 56 years of age reported by Clarke et al., contained no nemaline rods, which indicates that nemaline rods will not necessarily form in the muscle fibers of CFTD patients with *TPM3* mutations as they age.

In contrast to the homogeneity of pathological findings in our *TPM3*-related cases of CFTD, there is significant clinical and genetic heterogeneity in this disease, and in general, there were no significant clinical features that differentiated patients with *TPM3* mutations from other patients with congenital myopathy. The identified mutations exhibited three patterns of inheritance: autosomal dominant, autosomal recessive, and *de novo* (dominant) mutations. As reported in patients with *TPM3*-associated nemaline myopathy, presenting symptoms varied from neonatal hypotonia and delayed motor milestones in early childhood to difficulty with exercise in adolescence. Similarly, the degree of weakness varied from severe to clinically unrecognized, with the recessive mutations causing more significant impairment (Durling, et al., 2002; Laing, et al., 1992; Laing, et al., 1995; Lehtokari, et al., 2008; Penisson-Besnier, et al., 2007; Tan, et al., 1999; Wattanasirichaigoon, et al., 2002). The degree of clinical heterogeneity is exemplified by patient 126-1 and his mother, patient 126-2. Despite having the same heterozygous mutation, the mother's presentation was less severe than the child's to the point that her symptoms were only recognized retrospectively after her son's diagnosis. Although we cannot rule out the possibility of mosaicism in the mother, careful analysis of peak heights in the DNA sequence chromatograms revealed no noticeable differences between the tracings from mother and son. This clinical variability suggests the possibility of other genetic or environmental influences on the course of disease. Similarly, the presence of identical mutations in patients with nemaline myopathy and CFTD suggests that additional factors are responsible for the degree and type of disease seen in these patients. Unlike some previously described patients with *TPM3* mutations, the patients in our study were not reported to present with a significantly greater weakness of the lower limbs or foot drop as a prominent feature (Durling, et al., 2002; Laing, et al., 1995). However, since we did not personally examine all patients, we cannot exclude this possibility.

In total, mutations in three genes, *ACTA1*, *SEPN1*, and now *TPM3*, have been identified in cases of CFTD. Although there have been suggestions that CFTD is a "catch-all" diagnosis, useful only as a placeholder until a more specific diagnosis can be established, our data and the recent report by Clarke et al (Clarke, et al., 2008) demonstrate clearly that there exists a definable group of patients, with a consistent clinical and pathological presentation, associated with mutations of the *TPM3* gene. We identified *TPM3* mutations in 5 of 13 cases of CFTD, including 3 novel mutations. In addition, we discovered a *TPM3* mutation in 1 of 18 selected cases of nemaline myopathy. The spectrum of clinicopathological presentations associated with *TPM3* now includes both nemaline myopathy and CFTD, and given the fact that the *TPM3* gene product, α TM_{slow} is predominantly expressed in type 1 (slow) muscle fibers, this gene should be regarded as a prime candidate for other congenital myopathies in which type 1 fiber hypotrophy is a major pathologic finding. This report brings the total

number of unrelated individuals with reported *TPM3* associated congenital myopathy to 20, including 10 cases of CFTD, 7 of nemaline myopathy, 1 of cap disease, and 2 families with both CFTD or fiber size variation and NM in different members of the same family. While the usefulness of the CFTD diagnosis has been controversial (Clarke and North, 2003), these results support the utility of the CFTD diagnosis in directing the course of genetic testing and suggest that *TPM3* should be considered as the first line of testing in patients with clinical symptoms consistent with congenital myopathy and relatively homogeneous type 1 hypotrophy without other pathological features.

Acknowledgments

This work was supported by NIH (National Institutes of Arthritis and Musculoskeletal and Skin Diseases R01 AR044345), the Muscular Dystrophy Association of the USA, the Joshua Frase Foundation, and the Lee and Penny Anderson Family Foundation. DNA sequencing was performed by the Children's Hospital Boston Genomics core DNA sequencing facility (National Institute of Child Health and Human Development grant, P30HD18655).

The authors thank the many healthcare providers who contributed to this study, particularly Drs. Tom Crawford, Basil Darras, Peter Kang, Umberto De Girolami, Hart Lidov, Adnan Manzur, Eileen McCormick, E. Tessa Hedley-Whyte, and Francesco Muntoni, genetic counselors Nicole Dexter, Ana Morales, and Corinne Strickland, and research assistant Tanya Holmes, for their help for enrolling and obtaining samples and clinical and pathological data from these families, and Zoe Chen for assistance with *TPM3* screening. Special thanks to all the patients and families who participated.

References

- Clarke NF, Kidson W, Quijano-Roy S, Estournet B, Ferreiro A, Guicheney P, Manson JJ, Kornberg AJ, Shield LK, North KN. SEPN1: associated with congenital fiber-type disproportion and insulin resistance. *Ann Neurol*. 2006; 59(3):546–52. [PubMed: 16365872]
- Clarke NF, Kolski H, Dye DE, Lim E, Smith RL, Patel R, Fahey MC, Bellance R, Romero NB, Johnson ES, Labarre-Vila A, Monnier N, Laing NG, North KN. Mutations in TPM3 are a common cause of congenital fiber type disproportion. *Ann Neurol*. 2008; 63(3):329–37. [PubMed: 18300303]
- Clarke NF, North KN. Congenital fiber type disproportion--30 years on. *J Neuropathol Exp Neurol*. 2003; 62(10):977–89. [PubMed: 14575234]
- den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat*. 2000; 15(1):7–12. [PubMed: 10612815]
- Dubowitz, VSC. *Muscle Biopsy: A Practical Approach*. Philadelphia: Saunders; 2007.
- Durling HJ, Reilich P, Muller-Hocker J, Mendel B, Pongratz D, Wallgren-Pettersson C, Gunning P, Lochmuller H, Laing NG. De novo missense mutation in a constitutively expressed exon of the slow alpha-tropomyosin gene TPM3 associated with an atypical, sporadic case of nemaline myopathy. *Neuromuscul Disord*. 2002; 12(10):947–51. [PubMed: 12467750]
- Gunning PW, Schevzov G, Kee AJ, Hardeman EC. Tropomyosin isoforms: divining rods for actin cytoskeleton function. *Trends Cell Biol*. 2005; 15(6):333–41. [PubMed: 15953552]
- Ilkovski B, Mokbel N, Lewis RA, Walker K, Nowak KJ, Domazetovska A, Laing NG, Fowler VM, North KN, Cooper ST. Disease severity and thin filament regulation in M9R TPM3 nemaline myopathy. *J Neuropathol Exp Neurol*. 2008; 67(9):867–77. [PubMed: 18716557]
- Jaffe M, Shapira J, Borochowitz Z. Familial congenital fiber type disproportion (CFTD) with an autosomal recessive inheritance. *Clin Genet*. 1988; 33(1):33–7. [PubMed: 3342545]
- Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci*. 1973; 18(1):111–29. [PubMed: 4120482]
- Laing NG, Clarke NF, Dye DE, Liyanage K, Walker KR, Kobayashi Y, Shimakawa S, Hagiwara T, Ouvrier R, Sparrow JC, Nishino I, North KN, Nonaka I. Actin mutations are one cause of congenital fibre type disproportion. *Ann Neurol*. 2004; 56(5):689–94. [PubMed: 15468086]
- Laing NG, Majda BT, Akkari PA, Layton MG, Mulley JC, Phillips H, Haan EA, White SJ, Beggs AH, Kunkel LM, Groth DM, Boundy KL, Kneebone CS, Blumberg PC, Wilton SD, Speer MC,

- Kakulas BA. Assignment of a gene (NEMI) for autosomal dominant nemaline myopathy to chromosome 1. *Am J Hum Genet.* 1992; 50(3):576–83. [PubMed: 1347195]
- Laing NG, Wilton SD, Akkari PA, Dorosz S, Boundy K, Kneebone C, Blumbergs P, White S, Watkins H, Love DR, Haan E. A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. *Nat Genet.* 1995; 9(1):75–9. [PubMed: 7704029]
- Lees-Miller JP, Helfman DM. The molecular basis for tropomyosin isoform diversity. *Bioessays.* 1991; 13(9):429–37. [PubMed: 1796905]
- Lehtokari VL, Pelin K, Donner K, Voit T, Rudnik-Schoneborn S, Stoetter M, Talim B, Topaloglu H, Laing NG, Wallgren-Pettersson C. Identification of a founder mutation in TPM3 in nemaline myopathy patients of Turkish origin. *Eur J Hum Genet.* 2008; 16(9):1055–61. [PubMed: 18382475]
- Ohlsson M, Fidzianska A, Tajsharghi H, Oldfors A. TPM3 mutation in one of the original cases of cap disease. *Neurology.* 2009; 72(22):1961–3. [PubMed: 19487656]
- Penisson-Besnier I, Monnier N, Toutain A, Dubas F, Laing N. A second pedigree with autosomal dominant nemaline myopathy caused by TPM3 mutation: a clinical and pathological study. *Neuromuscul Disord.* 2007; 17(4):330–7. [PubMed: 17376686]
- Ryan MM, Ilkovski B, Strickland CD, Schnell C, Sanoudou D, Midgett C, Houston R, Muirhead D, Dennett X, Shield LK, DeGirolami U, Iannaccone ST, Laing NG, North KN, Beggs AH. Clinical course correlates poorly with muscle pathology in nemaline myopathy. *Neurology.* 2003; 60(4):665–73. [PubMed: 12601110]
- Tan P, Briner J, Boltshauser E, Davis MR, Wilton SD, North K, Wallgren-Pettersson C, Laing NG. Homozygosity for a nonsense mutation in the alpha-tropomyosin slow gene TPM3 in a patient with severe infantile nemaline myopathy. *Neuromuscul Disord.* 1999; 9(8):573–9. [PubMed: 10619715]
- Wattanasirichaigoon D, Swoboda KJ, Takada F, Tong HQ, Lip V, Iannaccone ST, Wallgren-Pettersson C, Laing NG, Beggs AH. Mutations of the slow muscle alpha-tropomyosin gene, TPM3, are a rare cause of nemaline myopathy. *Neurology.* 2002; 59(4):613–7. [PubMed: 12196661]

Figure 1.

(A): Schematic diagram of *TPM3* gene, indicating the 5 ubiquitously expressed exons (white), 5 muscle specific exons (black), and 4 non-muscle exons (gray). Exons are numbered according to Durling et al. (2002). Shown above the gene are previously described mutations associated with nemaline myopathy and/or CFTD (p.Stop286fsX73 was previously reported as p.X285NextX74 (Lehtokari, et al., 2008)). Shown below are the mutations identified in this study. The p.Arg168His mutation was identified in two of our patients, one with CFTD and one with nemaline myopathy. Dominant mutations are defined as any single heterozygous change, whether *de novo* or dominant in the family. All recessive mutations described thus far have been nonsense mutations or mutations that affected the stop codon, causing production of a larger protein. Previously, two different numbering schemes have been used for numbering the *TPM3* gene and protein: accession no. X04201 and no. NM_152263.2. In this report, we employed the current standard numbering scheme, starting from the first ATG for cDNA numbering and with the first methionine for amino acid numbering. Our reference sequence was the National Center for Biotechnology Information (NCBI) accession number NM_152263.2. (B) Western blot analysis for tropomyosins in the muscles of Patients 311-1, 313-1, and 343-1. Molecular weight differences allow the distinction between alpha tropomyosin (α Tm) and beta tropomyosin (β Tm). Patient 313-1 has an additional band approximately 6 kD larger than than normal α -tropomyosin that reflects the additional 57 amino acids incorporated into TPM3 as a result of the p.268Ser mutation, as previously reported (Wattanasirichaigoon, et al., 2002). MW: Molecular weight marker.

Figure 2.

Pathologic features of patients with a diagnosis of CFTD and a *TPM3* mutation (Patient 913-1 is shown) included type 1 fiber hypotrophy, in the absence of other pathologic findings, as seen on (A) H and E, (B) ATPase (at pH 9.4) and (C) NADH stains. A biopsy from one case with *TPM3* mutation and a diagnosis of nemaline myopathy (Patient 343-1) contained numerous nemaline rods within fibers, as shown on (D) H and E, (E) Gomori trichrome, and (F) toluidine blue staining of ultrathin sections. Bar = 100 μ m.

Table 1

Pathological data from selected patients in this study*

Patient #	Referring Diagnosis	TPM3 Mutation	% of type 1 fibers in biopsy	Fiber size proportion (Type 1/Type 2)	Additional Findings
126-1	CFTD	c.[=] + [11C>T]	51	0.53	
126-2	None	c.[=] + [11C>T]	57	0.67	
311-1	CFTD	c.[=] + [721G>A]	48	0.48	
313-1	CFTD	c.[857A>C]	40	0.29	
913-1	CFTD	c.[=] + [272G>C]	53	0.44	
247-4	CFTD	c.[=] + [503G>A]	N/A	N/A	
343-1	Nemaline myopathy	c.[=] + [503G>A]	73	0.48	Nemaline rods
579-1	CFTD	None	56	0.51	
140-1	CFTD	None	58	0.61	Dystrophic changes
668-1	CFTD	None	64	0.49	Central cores
895-1	CFTD	None	70	0.91	Neuropathic changes
903-1	CFTD	None	62	0.67	Fiber type grouping, mild increase in centrally- nucleated fibers (~10%)
901-1	CFTD	None	51	0.56	Subsarcolemmal mitochondrial accumulations and atrophy of both fiber types
924-3	CFTD	None	61	1.45	Type 2 fiber atrophy
858-1	CFTD	None	74	0.56	Rare nemaline rods

* Data are presented for all 13 cases with referring diagnoses of CFTD and the one nemaline myopathy patient with a *TPM3* mutation. Patient 126-2 was evaluated because she was the mother of 126-1, and had mild nonspecific weakness, but no formal diagnosis of CFTD. Slides from patient 247-1 were unavailable for pathological review and fiber measurement.

Table 2

Clinical and Molecular Findings in Patients with *TPM3* Mutations

Patient #	Clinical Diagnosis	Sex	Mutation	Amino Acid Substitution	Inheritance	Disease Severity	Presentation	Age at Biopsy/ Muscle biopsied	Respiratory Issues	Feeding Issues	Status
126-1	CFTD	M	c.[=] + [11C>T]	p.Ala4Val	AD	Mild	Presented with poor suck, slow feeding and hypotonia at birth. History of cryptorchidism, PDD/ADHD and verbal learning disorder. At 5.5 y has facial weakness, but relatively normal tone and strength.	16 m/quadrice ps	None	Poor feeding early in life	Alive and ambulatory/able to run at 4 y; Walked at 18 – 21 m
126-2	CFTD (Retrospectively)	F	c.[=] + [11C>T]	p.Ala4Val	AD	Mild	Diagnosed after son. Retrospectively recalls: always slow runner, poor strength, history of myalgia with exertion and distal parathesias. Premature ventricular contractions since 33 y. At 39 y she reports having ptosis, myopathic facies and diffuse mild weakness.	29 y/vastus lateralis	None	Occasional difficulty with swallowing and choking; Slow feeder	Alive and ambulatory at 39 y
311-1	CFTD	F	c.[=] + [721G>A]	p.Glu241Lys	De novo	Moderate	Presented with abnormal posture in utero and gross motor delay by 5 m. At 8.75 y has facial weakness, ptosis, dysarthria, diffuse muscle weakness (especially	5 m/quadrice ps	Required O2 for first few days of life and nocturnal Bi-pap from 8 y	None	Alive and ambulatory for short distances at 8.75 y; Walked without support at 5 y; Never ran

Patient #	Clinical Diagnosis	Sex	Mutation	Amino Acid Substitution	Inheritance	Disease Severity	Presentation	Age at Biopsy/ Muscle biopsied	Respiratory Issues	Feeding Issues	Status
313-1	CFTD	F	c.[857A>C] +[857A>C]	p.X286Ser	AR (distant consanguinity 3 generations removed)	Severe	Presented with axial), and lordosis. Presented with hypotonia, head lag, and motor delay by 3 m. LE initially weaker than UE. Marked pectus excavatum by 2.3 y. At 9.5 y has facial weakness, significant dysarthria, scoliosis, and severe weakness of upper and lower limbs.	1 y/quadrice ps	Ventilator dependent since 2.5 y	G-tube since 2.5 y; Minimal oral foods until at least 8 y.	Alive and non- ambulatory at 9.5 y (Never walked)
913-1	CFTD	F	c.[=] + [272G>C]	p.Arg91Pro	De novo	Too young	Presented with bifacial paresis and hypotonia by 2 m. At 14 m has dysarthria, no head/neck control, and moderate diffuse weakness.	6 m/vastus lateralis	Hx of neonatal tachypnea and seesaw respirations	Poor suck and poor weight gain in infancy	Alive and non- ambulatory at 14 m
247-4	CFTD	F	c.[=] + [503G>A]	p.Arg168His	De novo	Mild	Presented with neonatal hypotonia, delayed motor milestones, and joint laxity. At 18 y has myopathic facies, scoliosis, fatigue, difficulty with weight gain, finger/heel cord contractures, and mild to moderate weakness of axial/proximal muscles. Neck flexors and axial muscles	11 y/unreported	Nocturnal BiPAP since 12 y	Chewing and swallowing difficulties; Slow feeder	Alive and ambulatory/ able to run at 18 y (limited distance due to respiratory issues); Walked at 19 m

Patient #	Clinical Diagnosis	Sex	Mutation	Amino Acid Substitution	Inheritance	Disease Severity	Presentation	Age at Biopsy/ Muscle biopsied	Respiratory Issues	Feeding Issues	Status
343-1	NM	M	c.[=] + [503G>A]	p.Arg168His	AD (no known family history)	Mild	Diagnosed after respiratory failure at 58 y. Retrospectively recalls difficulty keeping up with peers in childhood. At 66 y had mild facial weakness, dysarthria, mild scoliosis/hyperlordosis, hypothyroidism, head drop, and mild weakness of axial, some proximal and some distal muscles.	59 y/biceps	Occasional nocturnal O ₂ since 58 y	Occasional difficulty chewing and swallowing	Ambulatory until death at 66.9 y of thyroid cancer; Wheelchair for long distances

Nucleotide number in bold indicates cDNA number with +1 corresponding to the A of the ATG translation initiation codon. GenBank accession NM_152263.2. AD = autosomal dominant; AR = autosomal recessive; De novo = indicates a sporadic mutation excluded in both parents. LE = lower extremity; UE = upper extremity; PDD = pervasive developmental delay; ADHD = attention deficit/hyperactivity disorder; y = years; m = month/s. BiPAP = bi-level positive airway pressure.