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Cap disease caused by heterozygous deletion of the β -tropomyosin gene *TPM2*

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Abstract

“Cap myopathy” or “cap disease” is a congenital myopathy characterised by cap-like structures at the periphery of muscle fibres, consisting of disarranged thin filaments with enlarged Z discs. Here we report a deletion in the β -tropomyosin (*TPM2*) gene causing cap disease in a 36-year-old male patient with congenital muscle weakness, myopathic facies and respiratory insufficiency. The mutation identified in this patient is an in-frame deletion (c.415_417delGAG) of one codon in exon 4 of *TPM2* removing a single glutamate residue (p.Glu139del) from the β -tropomyosin protein. This is expected to disrupt the seven-amino acid repeat essential for making a coiled coil, and thus to impair tropomyosin–actin interaction. Missense mutations in *TPM2* have previously been found to cause rare cases of nemaline myopathy and distal arthrogryposis. This mutation is one not previously described and the first genetic cause identified for cap disease.

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1. Introduction

The congenital myopathies include a wide spectrum of clinically, histologically and genetically variable neuromuscular disorders, some of them caused by mutations in genes for sarcomeric proteins [1]. They are defined on the basis of muscle weakness and structural abnormalities of the muscle fibres. “Cap

myopathy” or “cap disease” was first described in 1981 by Fidzianska and co-workers [2]. To date, no genetic cause has been published for this congenital myopathy. Here we report a heterozygous in-frame deletion removing one codon from exon 4 of the β -tropomyosin (*TPM2*) gene in a patient with cap myopathy.

Mutations in this gene are not new as causes of muscle disorders; we have previously published heterozygous mutations in *TPM2* in two families with initial diagnoses of nemaline myopathy [3], and a third mutation has been described in a patient with distal arthrogryposis [4]. A further *TPM2* mutation causing

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arthrogryposis was reported at the World Muscle Society Congress in 2006 [5]. The current paper establishes for the first time a common causative gene for cases of nemaline myopathy and cap myopathy.

The roles of tropomyosins in stabilising the thin (actin) filament of the sarcomere [6] and in regulating muscle contraction [7] have been well defined in skeletal muscle. The tropomyosins exist as coiled-coil homo- or heterodimers forming head-to-tail polymers, running along the length of the actin molecule [8–11]. They are encoded by four different genes; αTm , i.e., *TPM1* (OMIM 191010), βTm , i.e., *TPM2* (OMIM 190990), γTm , i.e., *TPM3* (OMIM 191030), and δTm , i.e., *TPM4* (OMIM 600317) [12], generating more than 40 different tropomyosin isoforms due to the use of different promoters or variable intragenic splicing [12–14]. The tropomyosin genes *TPM1*, *TPM2*, and *TPM3* are expressed in skeletal muscle encoding isoforms αTm_{fast} , βTm , and αTm_{slow} . *TPM2* is expressed in both slow, and, to a lesser extent, fast muscle fibres. When both α - and β -tropomyosins are expressed, $\alpha\beta$ -heterodimers are preferentially formed over $\alpha\alpha$ -homodimers, and $\beta\beta$ -homodimers are rare [15].

2. Patient and methods

2.1. Patient

This male patient was born to healthy parents with no family history of neuromuscular symptoms. He was moderately hypotonic at birth but otherwise his neonatal period was unproblematic. His milestones were delayed and he achieved walking at the age of 24 months. When first examined at the age of 11 years, he had myopathic facies, dysarthria, hyperlordosis, and small muscle bulk with generalised muscle weakness and hypotonia. He was able to walk on his toes but not on his heels. Electromyography was suggestive of muscle disease. Nerve conduction velocities and electrocardiography were normal. A muscle biopsy was performed in the vastus lateralis.

At the age of 33 years the patient was admitted to an intensive care unit because of acute respiratory insufficiency, hypoventilation, and nosocomial pneumonia. Cardiological investigations gave normal results. Chronic hypoventilation was diagnosed and BIPAP ventilation started, leading to a clear improvement in the patient's condition.

On neurological re-examination the patient had myopathic facies with very high-arched palate, ptosis, truncal obesity, and webbed neck (Fig. 1). Muscle bulk was small in the limbs, especially distally. In the upper limbs, slight muscle weakness was noted distally, and in the lower limbs, weakness was proximal as well as distal. The patient did not report any significant reduction in muscle strength since childhood. He was able to walk for a few hundred metres but had always been unable to



Fig. 1. Patient

run and had difficulties climbing stairs. Muscle magnetic resonance imaging (MRI) showed normal neck and shoulder girdle muscles (not shown). There was fatty infiltration of the paraspinal muscles while the pelvic girdle muscles (not shown) were normal, with the exception of slight fatty infiltration of the gluteus maximus muscles. There was diffuse fatty infiltration of the thigh (Fig. 2A) and distal leg muscles (Fig. 2B) with some sparing of the rectus femoris, gracilis and sartorius. Creatine kinase was normal. A second muscle biopsy of the extensor digitorum longus was taken.

When the patient was last re-examined at the age of 36 years, muscle testing using the MRC scale showed weakness of grade 4+/5 of the facial muscles and flexors of the knees, and 4/5 for the shoulder girdle, the ankle dorsiflexors and the extensors of the toes, while the remaining muscles showed strength within normal limits. The patient has an education in information technology but is unable to work because of his muscle weakness and respiratory compromise.

2.2. Histopathology

The first biopsy from the vastus medialis muscle at 12 years of age had been processed for routine

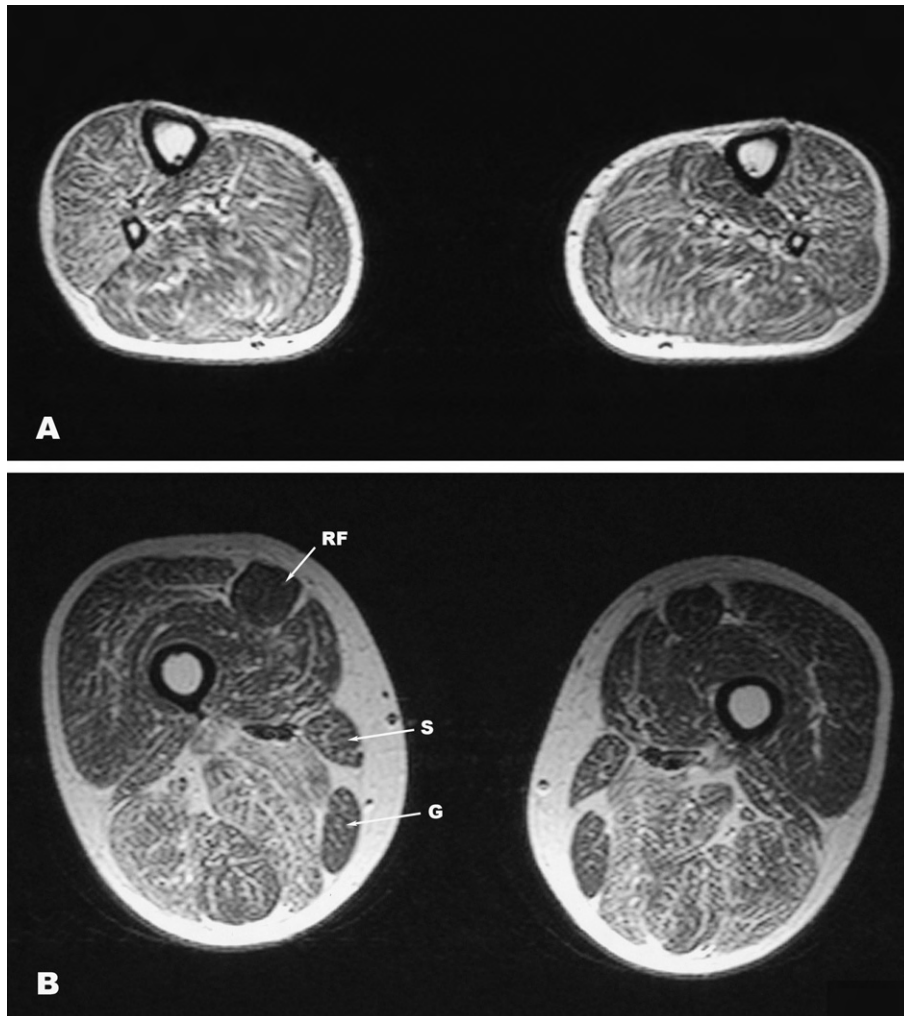


Fig. 2. MRI of the lower legs and thighs of the patient. (A) Leg muscles: diffuse fatty infiltration of all muscles. (B) Thighs: diffuse fatty infiltration prevalent in the hamstrings with lesser involvement of the gracilis (G), sartorius (S) and rectus femoris (RF) muscles.

histo-enzymology and electron microscopy (EM). No immunohistochemistry had been performed.

The second biopsy from the extensor digitorum longus was done at the age of 33 years and processed according to conventional procedures for histo-enzymology, immunohistochemistry, and EM. Technical data regarding source and working dilutions of antibodies used have been previously detailed [16,17]. Biopsy samples were too small for additional co-localisation analyses and immuno-EM.

2.3. Mutation analysis

DNA samples were available from the patient and his mother, but not from his father. Before proceeding to mutation analysis of *TPM2*, the genes for slow skeletal muscle actin, *ACTA1*, and for myotilin, were analysed by direct sequencing of the coding exons of these genes. No mutations were identified. We also analysed *TPM2* in DNA samples from an affected sib pair from a separate,

unpublished family with cap myopathy. Mutations in *ACTA1* had previously been excluded. Direct sequencing of the samples did not show any mutation in *TPM2*.

2.3.1. Polymerase chain reaction

To perform mutation screening for *TPM2* by sequencing we designed primers to amplify the exons expressed in the muscle isoform of the protein (exons 1A, 2B, 3+4, 5–6A, 6B+7, 8, 9A, and 9B), one or two exons at a time, in PCR reactions resulting in PCR products of 300–600 bp in length. Each 35 μ l reaction mix contained 90 ng of genomic DNA (3 μ l), 10 \times PCR buffer supplied with the AmpliTaq Gold containing 15 mM MgCl₂, 5 nmol each of dNTP, 20 pmol forward primer, 20 pmol reverse primer, and 0.8 U AmpliTaq Gold Polymerase Enzyme (Applied Biosystems, Roche Molecular Systems, Inc., Branchburg, USA). The reaction was carried out in a PTC-225 DNA Engine Tetrad Thermocycler (MJ Research, Waltham, USA) starting with denaturation for 10 min at 95 $^{\circ}$ C followed by



Fig. 3. Mutations located in the β -tropomyosin gene. Amino acids 100–159 are shown, as well as the corresponding cDNA sequence encoded by exon 3 (black) and exon 4 (blue). The novel mutation is shown in red and those published in Donner et al. [3] in pink. The seven amino acid repeats, abcdefg, characteristic for coiled-coil proteins are marked above the cDNA sequence. (DNA and amino acid sequences from Ensembl, <http://www.ensembl.org>, Ensembl transcript ID: ENST00000329305).

annealing at 58 °C, and extension at 72 °C. The lengths of the denaturation, annealing, and extension steps were 0.45 min. Final extension was performed at 72 °C for 10 min. Amplification of the PCR products was confirmed by agarose gel electrophoresis before sequence analysis.

2.3.2. Sequencing

The PCR products were purified using Exonuclease I and shrimp alkaline phosphatase (USB Corporation, Cleveland, USA) and the purified products were sequenced using BigDye version 3.1 sequencing chemistry and an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, USA). The sequences were analysed using Sequencher 4.5 software.

2.3.3. The coiled-coil structure analysis tool COILS

To further predict the effects of this mutation in the β Tm protein coiled-coil structure we used the tool COILS, version 2.2 available at www.ch.embnet.org/software/coils/COILS_doc.html. This tool is based on a prediction protocol by Parry and is described by Lupas et al. [18–20]. Using COILS we analysed the effect of the mutation described in this article,

and compared the result with the similarly analysed and predicted effects of the *TPM2* mutations we have previously published. These are a missense mutation Q147P in *TPM2* exon 4 and another missense mutation E117K in exon 3 (Fig. 3) [3]. Wild-type β Tm was used as a reference.

3. Results

3.1. Histopathology

The first biopsy (*M. vastus medialis*) showed fibre type disproportion with numerical preponderance and hypotrophy of type I fibres, and type II hypertrophy. Type IIb fibres were absent. A retrospective study, 20 years later, showed distinct cap-like lesions in type I muscle fibres.

The second biopsy (*M. extensor digitorum longus*) confirmed the fibre type disproportion with type I fibre atrophy and large type II fibres as well as cap-like myofibrillar lesions. These were mostly in type I fibres though some were in type II fibres (Fig. 4).

The histopathological characteristics of the cap-like lesions were similar in both biopsies and are summarised

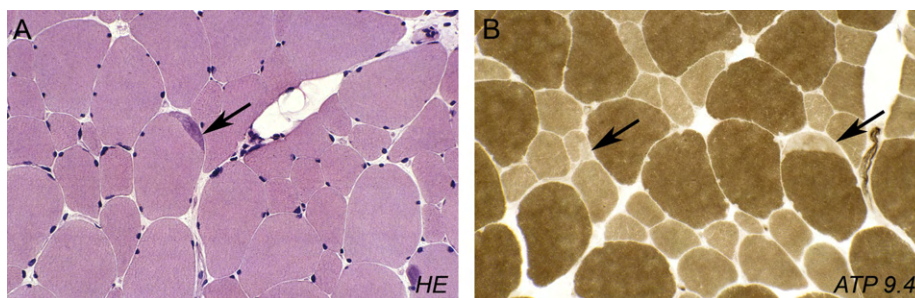


Fig. 4. Light microscopy (*M. extensor digitorum longus*). (A) HE stain showing fibre size disproportion with numerous hypotrophic and hypertrophic fibres. A cap-like structure is illustrated (arrow). (B) Myofibrillar ATPase staining at pH 9.4 revealing type I fibre type hypotrophy and type II fibre hypertrophy. A peripheral pale stained “cap” is seen in a type I (arrow, left) and in a type II fibre (arrow, right). Cryostat sections, 280 \times .

Table 1
Light microscopy: histology and histo-enzymology

STAINING	RESULTS
Haematoxylin and eosin	Eosinophilic (rarely basophilic)
Gomori trichrome	Dark green
Oil red O	Not detectable
Periodic acid Schiff	Not detectable
SDH	Not detectable
NADH-TR	Not detectable
M α GDH	Not detectable
Phosphorylases	Not detectable
AMPDeaminase	Absent
ATP pH 9.4, 4.6, 4.2	Pale

in Table 1. The observed immunoreactivity patterns of the caps are presented in Table 2 and a selection of the most obvious features are illustrated in Fig. 5.

By electron microscopy, the cap-like structures (Fig. 6A) consisted of partly modified subsarcolemmal areas of variable sizes showing different aspects. Disoriented disorganised myofibrils with lack of thick filaments and formation of Z-line extensions with a few short, rod-like structures (Fig. 6B) were often seen. More heterogeneous caps also showed several bundles of thin filaments, a few perpendicularly oriented myofilaments, glycogen particles, a few mitochondria and dilated sarcoplasmic reticulum (not illustrated). Slight Z-disc extensions were seen in many of the cross sections. Re-orientation of the blocks to obtain longitudinal sections rather showed “flame-shaped” Z-disc material. There was no accumulation of intermediate filaments. The sarcomeric organisation was well preserved but “jagged” (=thickened) Z bands were obvious in muscle fibre regions outside the caps. Non-specific concentric laminated bodies were numerous. Subsarcolemmal nuclei were normal.

3.2. Mutation analysis

By direct sequencing of a DNA sample of the patient concerned we identified an in-frame deletion, c.415_417delGAG (GenBank Accession No. NM_213674) in *TPM2* exon 4 causing the removal of one glutamic acid at position 139 in the protein (p.Glu139del, GenBank Accession No. NP_998839) (Fig. 3). The mother did not have the mutation, while the father was unavailable for study. This mutation was not detected in 200 control chromosomes analysed. We also screened samples from 20 additional probands with nemaline myopathy or other myopathies but found no further alterations in this gene.

The COILS tool predicted this mutation to disrupt the α -helical coiled-coil structure of the β Tm protein. This and the predicted effects of the mutations we identified previously are shown in the COILS output figures (Table 3 and Fig. 7). The wild-type β Tm was used as reference; the X-axis presenting the submitted amino acid sequence (from 1 to 260), and the Y-axis giving the probability for the correct coiled-coil structure. Thus, the reference amino acid sequence, i.e., wild-type β -tropomyosin, shows the probability value 1 along the entire length of the protein, i.e., given the present amino acid sequence, the likelihood that each of the amino acids is in its correct location. Comparing the mutated β -tropomyosin with the reference sequence COILS predicts the theoretical effect of the mutation on the protein structure. Using COILS we analysed the possible effect of the novel mutation described here, 139delE as well as those of the mutations Q147P in *TPM2* exon 4 and E117K in exon 3 previously identified by our group [3]. The 139delE mutation described in this paper is predicted to cause only a slight localised disruption of the coiled

Table 2
Light microscopy: immunohistochemistry: antibody expression patterns of caps: (immuno)reactive (R); nonreactive (NR)

Antigen	Results	Antigen	Results
Desmin	R	α B-Crystallin	NR
Ubiquitin	NR	α -Actinin	NR (rarely +)
Actin, sarcomeric A	R	SERCA1	NR
SERCA2	R	Titin	NR
Tropomyosin	R	Troponin T	R
Vinculin	NR	Nebulin	Strong R (clustered aggregates)
Telethonin	NR	MHCd, MHCn	NR
MHC-fast, slow	NR	Myosin	NR (rarely +)
Myotilin	R (rods)	Tubulin	NR
Vimentin	NR (rarely +)	DYS1,2,3	NR
SGL $\alpha,\beta,\gamma,\delta$	NR	Dysferlin	NR
Caveolin	NR	Spectrin	NR
Utrophin	NR	Merosin 80, 300kD	NR
4H8-2 (laminin α -2 chain)	NR	Laminin	NR
Emerin	NR	Lamins A/C	NR
Collagen VI	NR	τ -AT120	NR

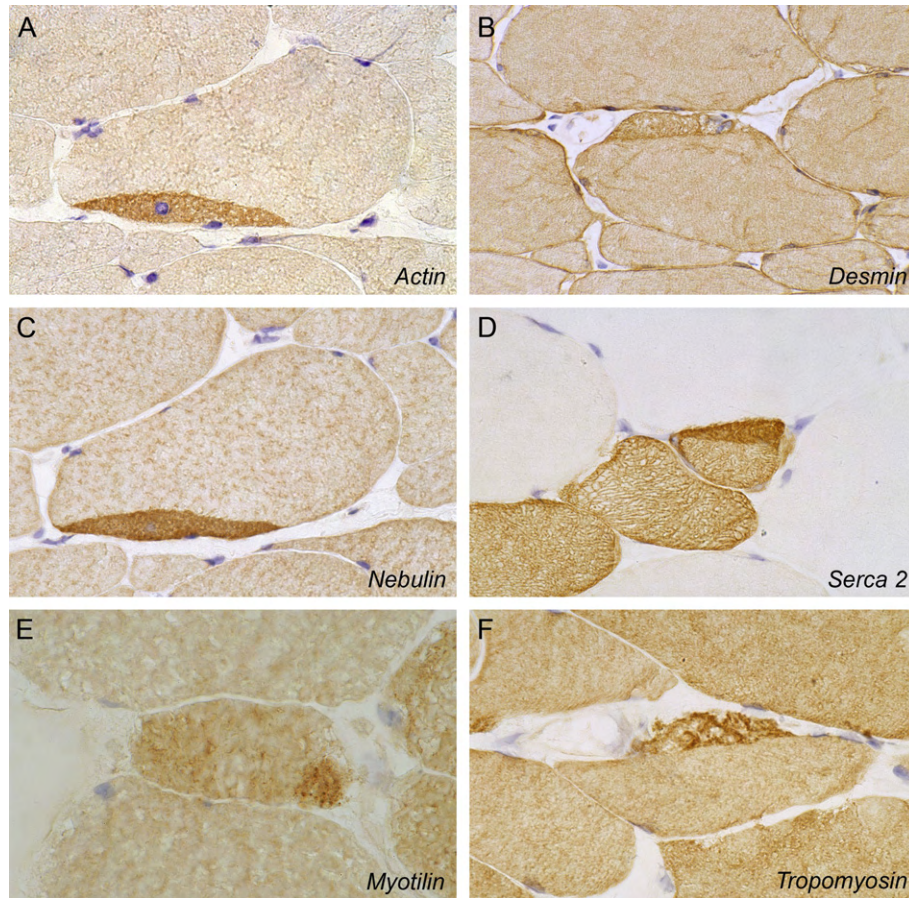


Fig. 5. Immunohistochemistry (*M. extensor digitorum*). Immunoreaction of caps for actin (A), desmin (B), nebulin (C), SERCA2 (D), myotilin (E), and tropomyosin (F). Note intense immunoreactivity for nebulin and an increased granular myotilin pattern. Cryostat sections, 728 \times .

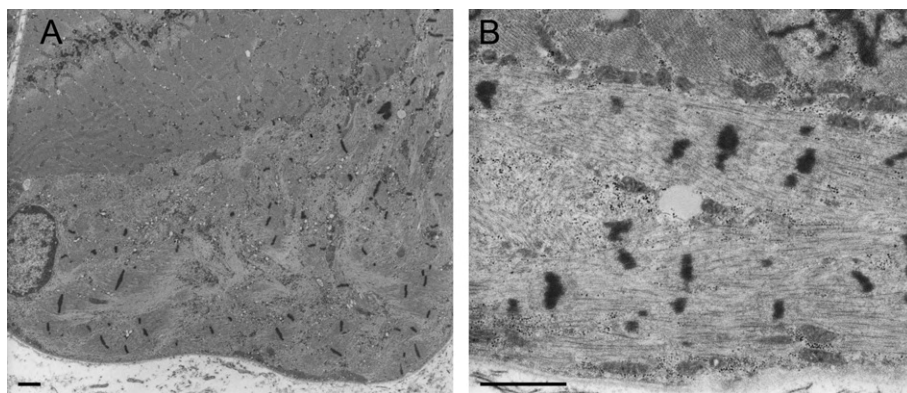


Fig. 6. Electron microscopy. (A) *M. vastus medialis* (first biopsy): general view of a large cap-like area characterised by a disorganised myofibrillar architecture and Z-disk abnormalities. Magnification: 5800 \times . (B) *M. extensor digitorum* (second biopsy); higher magnification of a cap-like lesion showing lack of thick myofilaments and slight rod formation. Magnification: 21,600 \times Standard techniques: fixation in glutaraldehyde, postfixation in osmium tetroxide, embedding in araldite, staining with uranyl acetate and lead citrate, examination with a Philips CM10 EM at 60 kV.

coil in the region spanning amino acids 138–154 (Table 3). The E117K mutation would have no effect while the Q147P mutation would have a major localised effect on the ability of the molecule to form a coiled coil.

4. Discussion

Tropomyosins are double-stranded coiled-coil proteins that bind to actin and cooperatively regulate contraction in striated muscle [15]. The tropomyosin

Table 3
COILS analysis result

Wild-type β -Tm				139delE				E117K				Q147P			
a.a. no	a.a.	rep	COILS	a.a. no	a.a.	rep	COILS	a.a. no	a.a.	rep	COILS	a.a. no	a.a.	rep	COILS
115	E	c	1.000	115	E	c	1.000	115	E	c	1.000	115	E	c	1.000
116	A	d	1.000	116	A	d	1.000	116	A	d	1.000	116	A	d	1.000
117	E	e	1.000	117	E	e	1.000	117	K	e	1.000	117	E	e	1.000
118	K	f	1.000	118	K	f	1.000	118	K	f	1.000	118	K	f	1.000
119	A	g	1.000	119	A	g	1.000	119	A	g	1.000	119	A	g	1.000
120	A	a	1.000	120	A	a	1.000	120	A	a	1.000	120	A	a	1.000
121	D	b	1.000	121	D	b	1.000	121	D	b	1.000	121	D	b	1.000
122	E	c	1.000	122	E	c	1.000	122	E	c	1.000	122	E	c	1.000
123	S	d	1.000	123	S	d	1.000	123	S	d	1.000	123	S	d	1.000
124	E	e	1.000	124	E	e	1.000	124	E	e	1.000	124	E	e	1.000
125	R	f	1.000	125	R	f	1.000	125	R	f	1.000	125	R	f	1.000
126	G	g	1.000	126	G	g	1.000	126	G	g	1.000	126	G	g	1.000
127	M	a	1.000	127	M	a	1.000	127	M	a	1.000	127	M	a	1.000
128	K	b	1.000	128	K	b	1.000	128	K	b	1.000	128	K	b	1.000
129	V	c	1.000	129	V	c	1.000	129	V	c	1.000	129	V	c	1.000
130	I	d	0.999	130	I	d	0.999	130	I	d	0.999	130	I	d	0.999
131	E	e	0.999	131	E	e	0.999	131	E	e	0.999	131	E	e	0.999
132	N	f	0.999	132	N	f	0.999	132	N	f	0.999	132	N	f	0.999
133	R	g	0.999	133	R	g	0.999	133	R	g	0.999	133	R	g	0.999
134	A	a	0.998	134	A	a	0.998	134	A	a	0.998	134	A	a	0.998
135	M	b	0.998	135	M	b	0.998	135	M	b	0.998	135	M	b	0.998
136	K	c	0.998	136	K	c	0.998	136	K	c	0.998	136	K	c	0.998
137	D	d	0.998	137	D	d	0.997	137	D	d	0.998	137	D	d	0.998
138	E	e	0.998	138	E	e	0.997	138	E	e	0.998	138	E	e	0.998
139	E	f	0.998	140	K	f	0.996	139	E	f	0.998	139	E	f	0.998
140	K	g	0.998	141	M	g	0.973	140	K	g	0.998	140	K	g	0.998
141	M	a	0.998	142	E	b	0.965	141	M	a	0.998	141	M	a	0.998
142	E	b	0.998	143	L	c	0.965	142	E	b	0.998	142	E	b	0.997
143	L	c	0.998	144	Q	d	0.965	143	L	c	0.998	143	L	c	0.997
144	Q	d	0.998	145	E	e	0.965	144	Q	d	0.998	144	Q	d	0.931
145	E	e	0.998	146	M	f	0.965	145	E	e	0.998	145	E	e	0.765
146	M	f	0.998	147	Q	g	0.965	146	M	f	0.998	146	M	f	0.357
147	Q	g	0.998	148	L	a	0.965	147	Q	g	0.998	147	P	g	0.117
148	L	a	0.998	149	K	b	0.965	148	L	a	0.998	148	L	a	0.924
149	K	b	0.998	150	E	c	0.965	149	K	b	0.998	149	K	b	0.924
150	E	c	0.998	151	A	d	0.965	150	E	c	0.998	150	E	c	0.957
151	A	d	0.998	152	K	e	0.965	151	A	d	0.998	151	A	d	0.957
152	K	e	0.998	153	H	f	0.965	152	K	e	0.998	152	K	e	0.957
153	H	f	0.998	154	I	g	0.966	153	H	f	0.998	153	H	f	0.957
154	I	g	0.998	155	A	a	0.998	154	I	g	0.998	154	I	g	0.966
155	A	a	0.998	156	E	b	0.998	155	A	a	0.998	155	A	a	0.998

a.a. no., the number of the amino acid; a.a., amino acid; rep, a–g heptapeptide repeat essential for making a coiled coil; COILS, the predicted effect on the protein coiled-coil structure using a window of 28 amino acid residues.

α -helical coiled coil consists of heptapeptide repeats (abcdefg in Fig. 7) where the a and d residues are generally non-polar and form the interhelical interface (or core) of the double-stranded structure [15]. The mutation described in this paper causes the omission of a single glutamate residue, p.Glu139del, which is at position *f* in the heptapeptide repeat. The mutation is similar to the removal of one lysine residue from the coiled-coil tail of the slow skeletal myosin heavy chain, causing distal myopathy of the Laing type [21]. Based on our theoretical prediction using the COILS tool [18–20], the p.Glu139del alteration in β -tropomyosin would cause only a slight disruption

of the coiled coil in the region spanning amino acids 138–154 (Table 3 and Fig. 7). Thus, the absence of an amino acid residue in this part of the β -tropomyosin polypeptide would interfere only minimally the formation of coiled-coil dimers. Thus disruption of the coiled-coil structure may not be the cause of the pathology. However, the deleted amino acid, Glu139, represents an outer surface acidic residue, which has been proposed to form close contact with actin [22]. Therefore, the mutation may impair tropomyosin-actin interactions. To study this further we have initiated actin-binding and tropomyosin dimerisation studies of the mutant β -tropomyosins identified.

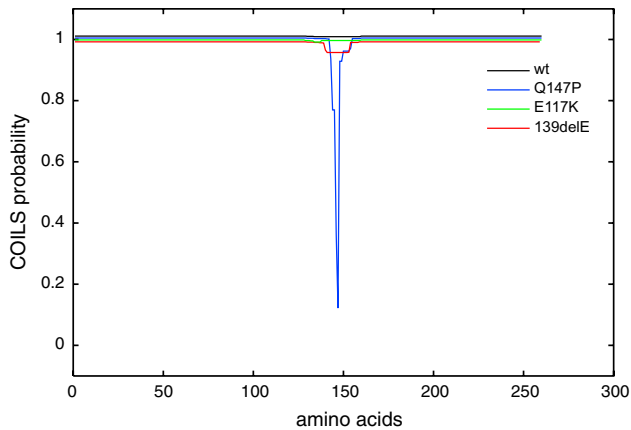


Fig. 7. Graphical illustration of COILS analysis results. Predicted theoretical effect of changes analyzed by COILS. Wild-type β Tm in black; 139delE in β Tm exon 4 in red; E117K in β Tm exon 3 in green; Q147P β Tm in exon 4 in blue.

This mutation is the first deletion observed in the *TPM2* gene; to date, all other mutations described in this gene have been heterozygous missense mutations [3–5]. To our knowledge, no deletions have been encountered in the *TPM3* gene either [23–25]; the mutations identified have been missense, nonsense and splice site mutations, or mutation of the termination codon.

The patient is the only affected person in the family, and heterozygous for this mutation, with no other mutation identified on sequence analysis of *TPM2* exons. His mother did not have the mutation, while the father was unavailable for study. Taken together, these *in silico* studies and the family data indicate that the mutation is likely to have arisen *de novo*, and to exert a dominant-negative effect.

Tropomyosins are a family of actin-binding proteins encoded by four different genes in humans, *TPM1–4*, of which *TPM1–3* are expressed in striated muscle tissue. More than 40 different tropomyosin isoforms are known, and their functions are well documented [26]. Studies in transgenic mice carrying a human-disease-causing mutation have shown that different tropomyosin muscle isoforms have different unique functional properties [27–29]. This may explain why mutations of tropomyosins can cause a variable range of muscle disorders: Mutations in *TPM1* encoding α Tm_{fast} which, in addition to striated muscles, is expressed in cardiac muscle, are known to cause cardiomyopathies [30–32]. Mutations in *TPM2* encoding β Tm and *TPM3*, the gene for α Tm_{slow}, have been reported to cause nemaline myopathy [3,23–25], while two groups have identified one mutation each in *TPM2* in patients with distal arthrogryposis [4,5]. The mutations identified to date in *TPM2* are expected also to alter the cytoskeletal tropomyosin isoform Tm1. Possibly, in non-muscle tissues, other tropomyosin isoforms compensate for the altered isoform.

Clinically, the patient has many features in common with the previously reported patients with cap myopathy [2,33–36] and also with patients with nemaline myopathy. A clinical and histological overlap between nemaline myopathy and cap myopathy can be noted in previous papers also [33,35,36]. Moreover, Cuisset and co-workers reported a family with two sibs affected by cap disease who had a half-cousin with nemaline myopathy [35]. Histologically, too, cap myopathy and nemaline myopathy have several features in common.

Clinical similarities with the typical form of nemaline myopathy include the relatively stable course and a similar distribution of muscle weakness, with facial weakness, high-arched palate, and inability to heel-walk. A further similarity is the respiratory compromise disproportionate to the weakness of muscles not used for respiration [34,37]. One difference is that our patient has ptosis, which is not very common in nemaline myopathy patients. In previous reports of cap myopathy, however, ptosis was not observed [2,33–35].

Furthermore, the MRI pattern of thigh muscle involvement is reminiscent of that seen in nemaline myopathy caused by nebulin mutations. One difference is that the rectus femoris, well preserved in this patient, is usually severely affected in nemaline myopathy [38–40]. The lack of neck flexor weakness, the weakness of the flexors but not the extensors of the knee, and the general body habitus and position of the patient is however different from that of the typical form of nemaline myopathy. In comparison with the patients with *TPM2* mutations described by Donner and co-workers, the patient described here also had no neck flexor weakness, and, as one of the patients in that report, was unable to walk on his heels [3]. Unlike the Dutch patient reported by Donner and co-workers, this patient showed no asymmetry of muscle weakness, but a histological similarity is that she also showed fibre type disproportion.

Histologically, the patient showed absence of type IIB fibres and smallness of type I fibres, features commonly encountered in nemaline myopathy and in other congenital myopathies also. The cap regions contained widened Z discs sometimes forming minute nemaline body-like structures. This is in concordance with previous reports of cap myopathy, where patients have had Z-disc abnormalities varying from “punctate” findings on trichrome staining and Z-disc widening to veritable nemaline bodies [2,33–36]. Such “punctate lesions” were also seen in one of the patients with *TPM2* mutations reported by Donner and co-workers, whose initial diagnosis was nemaline myopathy, but, who, when the biopsy was re-examined, turned out not to have nemaline bodies fulfilling the definition [3].

The caps were evenly positive for tropomyosin, actin, desmin, SERCA2, and troponin T, and strongly positive for nebulin also, while myotilin showed a coarse granular immunoreactivity, possibly reflecting stronger

labelling of the small nemaline body-like Z-disc extensions. Nemaline bodies, derivatives of the Z-discs and thin filaments, have previously been found to be immunoreactive for myotilin, actin, tropomyosin, and desmin [41]. We also found some ultrastructural similarities between our findings and those of nemaline myopathy, in which the nemaline bodies may be localised peripherally in disorganised sarcoplasmic areas. As in previous cases of cap myopathy, the caps were myosin-negative (not illustrated). In comparison with actinopathy patients [42], this patient had no large aggregates consisting of thin filaments only, nor intranuclear nemaline bodies.

Possibly, cap and nemaline body formation have similar mechanisms, through disruption of Z discs and thin filaments. This paper reports for the first time the identification of a genetic cause of cap myopathy, i.e., mutation of the β -tropomyosin gene, also known to cause cases of nemaline myopathy and distal arthrogryposis. Nemaline myopathy-causing mutations in this gene lead to nemaline bodies containing α -actinin and α -actin whereas this cap myopathy-causing mutation leads to both a separate location for the accumulated material and a difference in composition. The previously reported patient with distal arthrogryposis was not described as having specific structural alterations [4]. In other words, different mutations in *TPM2* can lead to very different structural abnormalities.

The fact that we did not identify any mutation of *TPM2* in the sib pair with cap myopathy suggests the existence of a separate gene for recessively inherited cap myopathy.

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