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SHORT COMMUNICATION

The R168G heterozygous mutation of tropomyosin 3 (TPM3) was identified in three family members and has manifestations ranging from asymptotic to serve scoliosis and respiratory complications

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Abstract According to existing reports, mutations in the slow tropomyosin gene (*TPM3*) may lead to congenital fiber-type disproportion (CFTD), nemaline myopathy (NM) and cap myopathy (CD). They are all congenital myopathies and are associated with clinical, pathological and genetic heterogeneity. A ten-year-old girl with scoliosis was unable to wean from mechanical ventilation after total intravenous anesthesia. The girl has scoliosis, respiratory insufficiency, motion delay and muscle weakness; her younger brother has a similar physiology but does not have scoliosis or respiratory insufficiency, and her parents are healthy. We conducted genetic testing and found a c.502C > G (p.R168G) heterozygous mutation in the family. This mutation originated from the father and was autosomal dominant. Muscle biopsy results indicated that no special structures were present, and the type I fiber ratio was not notably high compared to previous reports. Although the family members have the same mutations, their clinical manifestations are quite different.

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Introduction

Mutations in TPM3 may cause diseases, such as congenital fiber-type disproportion (CFTD), nemaline myopathy and cap myopathy.[1–3] These three kinds of myopathies are congenital myopathies (CMs), and they have similar clinical manifestations: high palate, narrow face, long face, motion delay, myasthenia, respiratory insufficiency, scoliosis, etc.[4] The onset time and severity of these illnesses vary; some patients have mild to severe systemic muscle weakness at birth or in the first year after birth, and some patients may die within two years in severe cases. However, in relatively mild cases, the patients manifested delayed movement; although these patients could walk and run, there was still a decrease in activity endurance and muscle strength compared with normal peers.[5]

Tropomyosin, which is encoded by TPM1, TPM2, TPM3 and TPM4, is an actin binding protein. It is a member of the tropomyosin family that encodes actin binding proteins. Tropomyosin is an alpha-helical coiled-coil heterodimer extending along seven actin subunits that provides stability to actin filaments and regulates the entry of other actin binding proteins.[6] In skeletal muscles, TPM3 only encodes α-tropomyosinslow, and its expression products are type I muscle fibers, which are slow twitch fibres.[7] The family mutation we found in this study is the c.502C > G (p.R168G) heterozygous mutation in exon 5. We identified 28 specific pathogenic sites of TPM3 mutations in the Human Gene Mutation Database (HGMD) professional database (Table 1). Thirty-eight mutations were recorded in the Leiden open mutation database at Leiden University Medical Center and Human Clinical Genetics Center, and some of the 38 mutations were variants of unknown significance (VUS) and benign. The c.502C > G (p.R168G) heterozygous mutations in this family clearly have clinical and pathological heterogeneity.

Materials and methods

This proband (a ten-year-old girl) with rigid scoliosis was treated with halo-pelvic traction. However, she could not wean from mechanical ventilation after total intravenous

Table 1 Currently identified TPM3 mutations based on HGMD.

Genetic coordinates	Protein change	DNA change	Reference
1:154145610-154145610	Leu149Ile	c.445C>A	Schreckenbach,et al.Neuromuscul Disord,24,117,2014
1:154164469-154164469	Met9Lys	c.26T>A	Park,et al.Muscle Nerve,58,235,2018
1:154142918-154142918	Arg245Gly	c.733A>G	Clarke,et al.Ann Neurol,63,329,2008
1:154145448-154145448	Arg168Gly	c.502C>G	Clarke,et al.Ann Neurol,63,329,2008
1:154148697-154148697	Arg91Cys	c.271C>T	Marttila,et al.Hum Mutat,35,779,2014
1:154142893-154142893	Thr253Lys	c.758C>A	Marttila,et al.Hum Mutat,35,779,2014
1:154164484-154164484	Ala4Val	c.11C>T	Lawlor,et al.Hum Mutat,31,176,2009
1:154140414-154140414	Term286Ser	c.857A>C	Wattanasirichaigoon,et al.Neurology,59,613,2002
1:154164401-154164401	Gln32Term	c.94C>T	Tan,et al.Neurobiol Dis,9,573,1999
1:154143170-154143173	del 3 bp codon 219	c.657_659delAGA	Donkervoort,et al.Ann Neurol,78,982,2015
1:154145589-154145589	Ala156Thr	c.466G>A	Kiphuth,et al.J Neurol,257,658,2010
1:154145429-154145429	Glu174Ala	c.521A>C	Munot,et al.Neuromuscul Disord,20,796,2010
1:154145445-154145445	Lys169Glu	c.505A>G	Clarke,et al.Ann Neurol,63,329,2008
1:154148705-154148705	Ser88Phe	c.263C>T	Marttila,et al.Hum Mutat,35,779,2014
1:154148670-154148670	Leu100Val	c.298C>G	Marttila,et al.Hum Mutat,35,779,2014
1:154164469-154164469	Met9Arg	c.26T>G	Laing,et al.Nat Genet,9,75,1995
1:154145432-154145432	Ile173Thr	c.518T>C	Stehl?kov?,et al.Clin Genet,91,463,2017
1:154148670-154148670	Leu100Met	c.298C>A	Clarke,et al.Ann Neurol,63,329,2008
1:154145448-154145448	Arg168Cys	c.502C>T	Clarke,et al.Ann Neurol,63,329,2008
1:154148696-154148696	Arg91Pro	c.272G>C	Lawlor,et al.Hum Mutat,31,176,2009
1:154140417-154140417	IVS11 as G-A -1	c.855-1G>A	Wattanasirichaigoon,et al.Neurology,59,613,2002
1:154142917-154142917	Arg245Ile	c.734G>T	Marttila,et al.Hum Mutat,35,779,2014
1:154142930-154142930	Glu241Lys	c.721G>A	Lawlor,et al.Hum Mutat,31,176,2009
1:154143154-154143157	del 3 bp codon 225	c.673_675delGAA	Donkervoort,et al.Ann Neurol,78,982,2015
1:154140415-154140416	del 2 bp codon 284	c.855delA	Lehtokari,et al.Eur J Hum Genet,16,1055,2008
1:154145447-154145447	Arg168His	c.503G>A	Penisson-Besnier,et al.Neuromuscul Disord,17,330,2007
1:154145603-154145603	Glu151Ala	c.452A>C	Marttila,et al.Hum Mutat,35,779,2014
#NA:#NA-#NA	deletion ex. 1-3		Iglesias,et al.Genet Med,16,922,2014

Table 2 The clinical features of the four members of the affected family.

Family members (age)	BMI (kg/m^2)	Age at walking (mo)	Echocardiography	CK (U/L) (40—100)	EMG	NCV	Pulmonary function	Clinical manifestations
Father (30 y)	19.5	12	Normal	66.5	Normal	Normal	Normal	Normal
Mother (29 y)	16.6	12	—	—	—	—	—	Normal
Daughter (10 y)	14.8	14	Tricuspid and slight pulmonary regurgitation	46	F wave of left peroneal nerve was not induced	Normal	FEV1 and PEF decreased slightly, FVC decreased severely	High palate; long face; motion delay; myasthenia; respiratory insufficiency; scoliosis; neck flexor weakness
Son (8 y)	12.5	13	Normal	63	Normal	Normal	Normal	Long face; motion delay; neck flexor weakness

anesthesia. After tracing her family, we found that her younger brother was physically similar to this poroband and had mild symptoms, her parents were both healthy. We performed genetic testing on this family and found that the TPM3 gene was mutated. Then, we performed muscle biopsy for the three affected members. The study was approved by the local ethics committees. Molecular investigations and muscle biopsies were performed with informed consent from the participating family members. The clinical data of the four family members are shown in Table 2.

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures using QIAamp DNA Blood Mini Kits (Qiagen, Germany). Thereafter, 3 μg of genomic DNA was fragmented by a Covaris sonicator (Covaris S2, USA) to sizes of 150—300 bp and then purified. The blunt ends of the purified DNA fragments were then repaired, and A-tailing was added. The fragments were ligated overnight using a standard Illumina paired-end (PE) adaptor. The ligated products were then amplified through 7-cycle polymerase chain reactions (PCRs) using PE primers containing 8 bp index tags. The purified PCR products containing 0.003 mg DNA were hybridized to the GenCapTM probe solution (Mygenostics Co. Ltd., China) at 65 °C for 22 h using a PCR thermocycler. The products were bound to a rotator for 1 h at room temperature using Dynal Myone Streptavidin C1 magnetic beads (Invitrogen, USA), which had been activated beforehand, and the products were then washed with buffer according to the kit manual. The captured DNA libraries were amplified using 15-cycle PCRs, purified, and subsequently eluted in a 0.03 ml volume and subjected to analysis on the Agilent 2100 Bioanalyzer and quantitative PCR to estimate the magnitude of enrichment. The final captured DNA libraries were sequenced using the Illumina HiSeq 2500 DNA Sequencer as PE 90 bp reads (following the manufacturer's standard cluster generation and sequencing protocols), providing an average coverage depth for each sample of at least 100-fold.

Image analysis, error estimation, and base calling were performed using the Illumina pipeline (version 1.3.4) with default parameters. Indexed primers were used to identify the different samples in the primary data. All unqualified reads (defined as reads either polluted by adaptors, containing more than 10% nucleotides out of read length, having an average quality of less than 10, or having 50% bases with a quality value less than 5) were removed using a local dynamic programming algorithm. The remaining reads were aligned to the reference human genome (UCSC hg19) using the Burrows-Wheeler Alignment tool (BWA-0.7.12-r1044). Next, SNPs and indels were identified using GATK software 3.4—46 using the recommended parameters. The depths of each region of a gene in different samples within the same sequencing lane are significantly correlated ($r > 0.7$), and the depth of each capture region was therefore used to calculate a z-score equation. The large deletions and duplications were identified using a predefined cut-off point (± 3) of the derived z-score of each captured gene region. We used the cut-off value of 3 for absolute z-score, as it represents the 99.9th percentile of the normal samples set for one tailed region. Any region with

a z-score above 3 was defined as either a deletion (<3) or a duplication (>3).

We performed muscle biopsies in the 3 affected members, and all specimens were taken from the biceps. The muscle was divided for serial frozen and paraffin-embedded sections, which were stained according to standard methods. We used light microscopy to observe muscle specimens subjected to histological staining and specialty staining. For electron microscopy, the muscle specimens were fixed in glutaraldehyde, postfixed in osmium tetroxide and embedded with epoxy resin according to routine processing protocols.

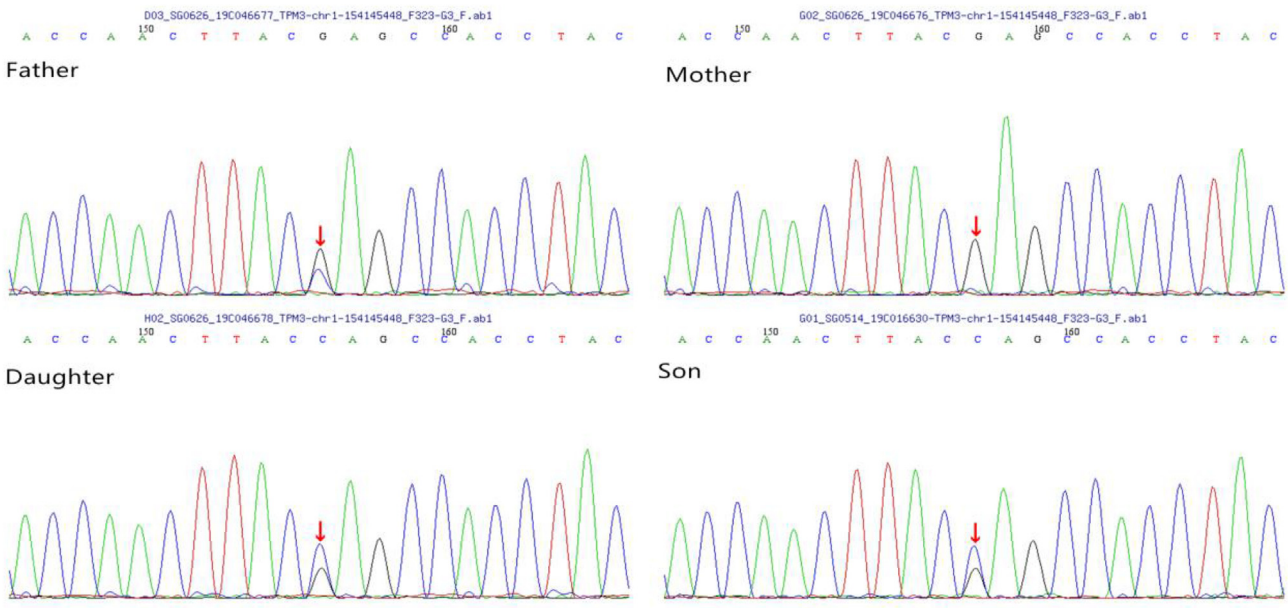

Figure 1 The DNA analysis results. The proband and her younger brother and her father all had a heterozygous mutation, and her mother was normal.

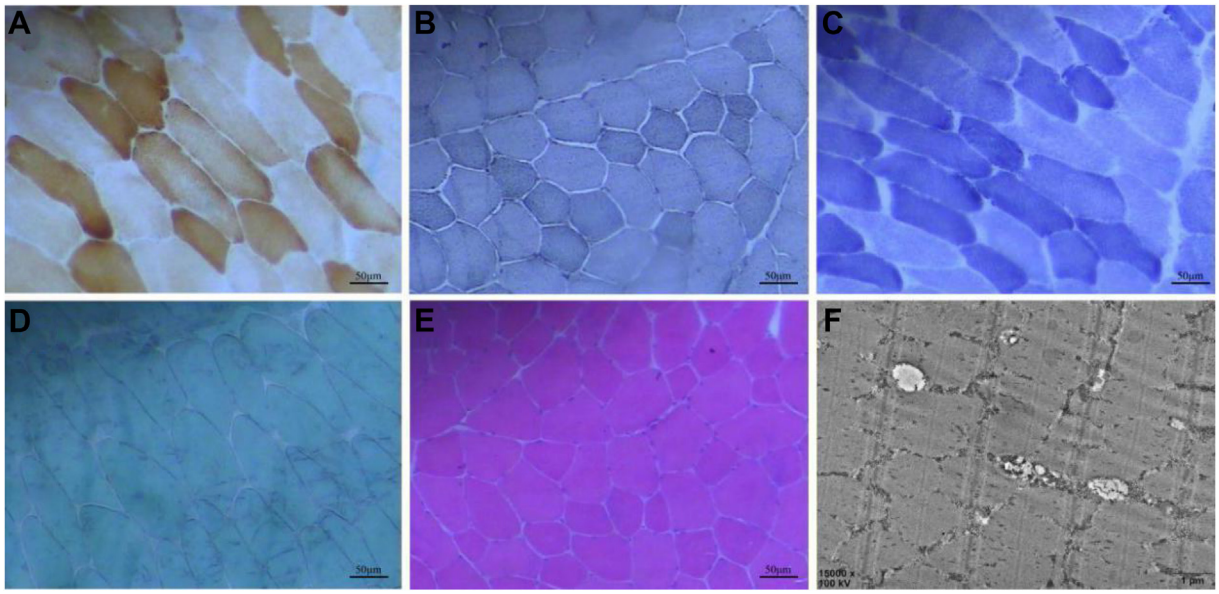

Figure 2 The muscle biopsies of the father. (A—E) Muscle biopsies stained by CCO, SBB, SDH, GT and H&E were observed by light microscopy (50 μm). **(A)** COX enzymes were normal. **(B)** Sudan black B (SBB) staining showed normal lipid composition in the muscle fibers. **(C)** SDH enzymes were normal. **(D)** No broken red fibers (RRF) were observed in Gomori staining. **(E)** No obvious degeneration, necrotic muscle fibers and regenerated muscle fibers were observed in H&E staining, and the type I fiber to type II fiber ratio was 1:2. **(F)** Muscle biopsies were observed by electron microscopy (1 μm). The sarcomeric structure was normal, and many glycogen deposits were observed.

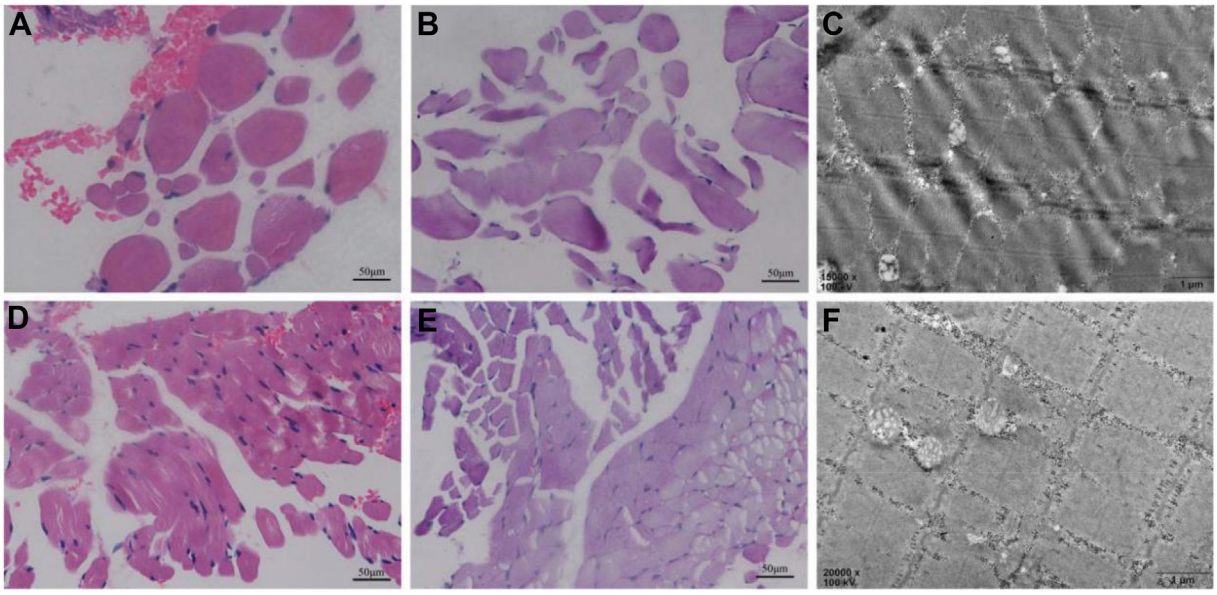

Figure 3 **(A-C)**: Muscle biopsies of the younger brother; **(D-F)**: muscle biopsies of the girl. H&E **(A)** and periodic acid Schiff (PAS) glycogen staining **(B)** showed some muscle fiber atrophy, and the type I to type II fiber ratio was 2:3. H&E **(D)** and periodic acid Schiff (PAS) glycogen staining **(E)** showed no obvious muscle fiber atrophy, and the type I to type II fiber ratio was 1:3. Many glycogen deposits were observed under an electron microscope **(C and F)**, but the sarcomeric structure was normal, and no special structures were observed.

Results and discussion

The genetic testing results showed that the father, son and daughter in the family all had the TPM3 c.502C > G (p.R168G) heterozygous mutation (Fig. 1), which originated from the father and was autosomal dominant. Muscle biopsies showed that the type I fiber and type II fiber ratio differences were not large. The type I fiber to type II fiber ratio was 1:2 for the father, 1:3 for the girl and 2:3 for her younger brother. Electron micrographs of muscle samples from the three affected members showed normal sarcomeric structure. Many glycogen deposits were observed under an electron microscope in the three members, but no cap structures or rod bodies were observed (Figs. 2 and 3).

Tropomyosin mutations are associated with most skeletal muscle myopathies, and the most common myopathy with TPM3 mutations is CFTD.[8–10] R168G is the high-frequency site of mutation. The molecular mechanisms are so complex that they have not been fully described. Tropomyosin can be described as a classical model [a-b-c-d-e-f-g].[11] R168G (f) is located on the surface of the tropomyosin dimer, this position can be used to bind other proteins. Mutations lead to muscle dysfunction and affect affinity with actin, changing filament length, Ca^{2+} sensitivity, and cross-bridge circulation.[12–17] Clarke et al[3] and Fidzianska et al[8] each reported one patient with the same site, but muscle biopsies showed some different details. Clarke et al reported that the type I fiber ratio was 95%. Fidzianska et al found cap structures in the biopsy, but the fiber proportion was not described. We did not find special structures, and the type I fiber ratio was not high in our biopsies. Muscle biopsies of patients at different ages may have different results.[18,19] Through our biopsies and clinical manifestations, there is no significant relationship between the mutation site and the severity of myopathy.

Usually, respiratory muscle weakness is common in many myopathies. In this family, the proband also suffered respiratory problems. Some physical exercises have been shown to improve respiratory muscle and lung function in children.[20] In addition, the use of a non-invasive ventilator is a good auxiliary measure.[21] These methods were used when treating the girl to improve her respiratory function, and she benefited from it. For most patients with myopathy, use of total intravenous anesthesia is safe to avoid anesthesia-related rhabdomyolysis. We suspected that the reason why this girl could not wean from mechanical ventilation was the abnormal metabolism of anesthetic drugs. Preoperative visits and examinations of anesthesia are very important. During the perioperative period, cardiopulmonary function, the level of muscle paralysis and the core temperature are worth monitoring.[22,23] Though the gene therapy of neuromuscular diseases has great potential and much research is undergoing, there is still no reliable treatment for congenital myopathy caused by genetic mutations because of the low incidence of disease, complex pathogenesis and very few beneficiaries.[24,25] The rapid development of gene sequencing has led to the discovery of many new genes that cause diseases. Through the inheritance mode of diseases, we can provide genetic counselling for affected families.[26]

In conclusion, we have identified TPM3 c.502C > G (p.R168G) mutations in a family, and three members were affected. Even in the same family with the same mutation, the severity of disease varies among members. Though we could identify the mutation, the exact mechanism and

progression of the disease should be thoroughly researched to guide therapy.

Conflict of Interests

The authors declare no conflicts of interest.

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