

Original Article

Heterogeneous phenotype detection in a Charcot-Marie-Tooth disease type 2A family with Mitofusin 2 gene Q751X mutation by targeted next-generation sequencing

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Abstract: Charcot-Marie-Tooth disease (CMT) is a group of clinically and genetically heterogeneous inherited neuropathies, characterized by slowly progressive distal weakness, wasting and sensory loss. CMT type 2A (CMT2A), caused by the mutations in the mitofusin 2 gene (*MFN2*), is the most common CMT2 subtype. Herein, we described the clinical, electrophysiological and pathological findings on a 4-generation family with four patients affected with CMT2A. Using targeted next-generation sequencing (NGS), a nonsense mutation Q751X in the *MFN2* gene was identified in the four patients. The clinical features, including onset age and phenotype severity, greatly varied among the patients in this family. One male member had a late disease onset and mild phenotype due to the heterozygous Q751X mutation, which was previously reported to be only associated with an early onset and severe CMT2A phenotype.

Keywords: Charcot-Marie-Tooth disease, *MFN2*, electromyography, sural nerve biopsy, next-generation sequencing

Introduction

Charcot-Marie-Tooth disease (CMT), also referred to hereditary motor and sensory neuropathy, is characterized by slowly progressive distal weakness, wasting and sensory loss. The prevalence of CMT is 1 in 2500 [1]. CMT is a genetically and clinically heterogeneous disorder. Almost 80 genes are currently known to cause CMT and related disorders [2]. CMT can be divided into a demyelinating form (CMT1) and an axonal form (CMT2) according to the inheritance pattern and electrophysiology [3]. CMT with an intermediate type has also been described [4]. CMT2 accounts for 20% of patients, and CMT2A is the most common subtype of CMT2 [5]. *MFN2* gene has been identified to be responsible for CMT2A. It encodes a protein Mitofusin-2, which is a GTPase embedded in the outer membrane of the mitochondria.

Generally, patients in different populations with *MFN* mutations of CMT2A either have an early onset and severe phenotype or a late onset and benign clinical course [5, 6]. There are very few reports on the clinical features of Chinese CMT2A patients with *MFN* mutations. Recent research suggested that the common characteristics of Chinese pedigree are early disease onset and moderate phenotypes [7]. Herein, we described a Chinese Han family with CMT2A caused by Q751X mutation in the *MFN2* gene. This mutation was previously reported to be only associated with an early onset and severe phenotype of CMT2A [7]. This family is unique because of inter-familial phenotypic variability. One male member showed late onset and mild phenotype, while two other members showed early onset and severe symptoms. This characteristic has never been reported.

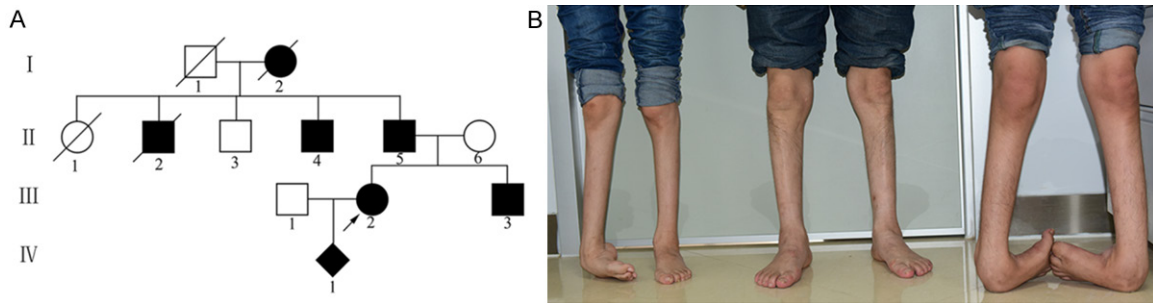


Figure 1. Pedigree and photograph of the family. A. The arrow in pedigree shows proband in this family. Squares represent males; circles represent females; rhombus represent fetus; white figures symbolize not affected individuals; black figures indicate individuals with CMT. B. The muscle atrophy of distal lower limbs and foot deformity, individual III-2, II-5, III-3 (from left to right).

ed in CMT2A Chinese Han family with *MFN2* mutations.

Material and methods

A Chinese Han 4-generation family including three symptomatic members and one fetus was enrolled in this study. One patient (**Figure 1A**, II-4) refused to participate in this study. Three symptomatic members underwent clinical examinations, including neurological examination and electromyography testing. One patient (**Figure 1A**, II-5) underwent the sural nerve biopsy. Informed consent was obtained from all individuals participating in this study. The CMT neuropathy scores (CMTNS) were used to evaluate the health impairment and CMT disease progression [8]. This study was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University (Changsha, China). Informed consent was obtained from all individual participants included in the study (Ethical approval number: 2014-S046).

Blood samples were drawn from five members of this family (**Figure 1A**, II-3, II-5, II-6, III-2, III-3). Targeted next-generation sequencing (NGS) of 63 disease-causing genes panel associated with known CMT and related disorders was performed on the index patient and 88.61% of target bases were covered to a total depth of $>20\times$ with high quality (Q20) reads. The reads were aligned with the human genome reference sequence [University of California Santa Cruz, human genome assembly 19 (UCSC hg19)]. Variants [single nucleotide polymorphisms (SNPs) and indels] were called with vcftools of the SaMTools software version 0.1.16 [9]. High

VarQuality SNPs were annotated with Perlscript into functional categories such as missense, nonsense, splice sites, coding, non-coding, and UTRs. Amino acid substitution (if it affects protein function) was annotated with SIFT (<http://sift.jcvi.org/>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>). The pathogenicity of variants was interpreted according to ACMG guidelines [10].

To confirm the potential mutation found by targeted NGS and segregation study, Sanger sequencing was performed on the family members. PCR amplifications were performed with forward primer [5'-CCTGGCGGGTAGTCCTAATA-3'] and reverse primer [5'-GGGTGCTTCATTCTCTTGGC-3']. PCR products were checked on agarose gels and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing results were compared with gene reference sequences in the UCSC to confirm potential mutations.

Prenatal diagnosis was conducted on the proband's fetus. Chorionic villi samples were collected under ultrasonic guidance at 10 weeks of gestation. Genomic DNA was extracted from the samples and genetic testing was performed by Sanger sequencing. Thus, the mutations in the fetus were detected.

Results

Clinical features

There are four generations of this family, including seven affected individuals with an autosomal dominant pattern of inheritance (**Figure 1A**). The clinical features of these patients dif-

Table 1. Clinical features of the CMT2A family

Patients	Sex/Age	Onset age	Symptoms	Strength		Pinprick	Vibration	Reflexes	CMTNS
				UL	LL				
III-2	F/25	2	Difficulty walking and foot deformity, Hand weakness	3/5	3/5 planter flexion, 0/5 Dorsiflexion, 3/5 quadriceps femoral	Severely reduced below knee/elbow	Severely reduced below knee/elbow	Reduce	27
III-3	M/21	3	Wheelchair, Feet deformity, Hand weakness	3/5	0/5 planter flexion, 0/5 dorsiflexion, 4/5 quadriceps femoral	Severely reduced below knee/elbow	Severely reduced below knee/elbow	Reduce	27
II-5	M/46	30 s	Abnormal gait	N	4/5 distal muscles	Mild reduced below ankle	Mild reduced below ankle	Sluggish in lower extremities	7

Abbreviations: CMT2A = Charcot-Marie-Tooth disease type 2A; N = not observed; CMTNS = CMT neuropathy score.

Table 2. The electromyography results of the family

	Normal range	III-2	III-3	II-5
Motor				
Median n.				
CMAP (mV)	≥5.0	0.17	0.27	1.44
NCV (m/s)	≥45.0	42	44	51.4
Ulnar n.				
CMAP (mV)	≥5.0	0.68	0.14	1.9
NCV (m/s)	≥45.0	44.3	32.75	34.5
Peroneal n.				
CMAP (mV)	≥3.0	Absent	Absent	Absent
NCV (m/s)	≥40.0	Absent	Absent	Absent
Tibial n.				
CMAP (mV)	≥3.0	Absent	Absent	Absent
NCV (m/s)	≥40.0	Absent	Absent	Absent
Sensory				
Median n.				
SAP (μV)	≥3.0	2.6	10.6	13.6
NCV (m/s)	≥45.0	31.7	32.2	33.2
Ulnar n.				
SAP (μV)	≥3.0	3.6	3.38	21.3
NCV (m/s)	≥44.0	30	31.5	51.2
Sural n.				
SAP (μV)	≥3.0	Absent	Absent	Absent
NCV (m/s)	≥40.0	Absent	Absent	Absent

Abbreviations: CMAP = compound muscle action potential; NCV = Nerve Conduction Velocity; SAP = sensory action potential.

ferred significantly (**Table 1**). The proband was a 25-year-old female with eight weeks of pregnancy. She had lower limbs weakness and difficulty in walking since two years of age. When she was six years old, she had apparent muscle atrophy of distal lower extremities, right foot with high arches and hammer toes, and had to use orthotic footwear (**Figure 1B**, III-2). Her

intrinsic hand muscles gradually atrophied at eight years of age, which made it difficult to complete fine movements. Neurological examination of the proband showed apparent muscle weakness of lower limbs and distal upper limbs. Deep tendon reflex was reduced in all extremities. Sensations of touch, pain and vibration were prominently reduced in both upper and lower limbs. The electromyography results are summarized in **Table 2**. It revealed that motor nerve conduction velocities (MNCVs) of median nerve were slightly decreased. They were consistent with an axonal polyneuropathy. Her CMT Neuropathy Score (CMTNS) was 27. Her 21-year-old younger brother also had severe symptoms (**Figure 1B**, III-3). He had distal limb weakness and difficulty in walking from three years of age, and was unable to walk because of foot deformity at eight years. His clinical examination and electromyography results were similar to proband (**Tables 1** and **2**). His CMTNS was also 27.

Although the sister and brother showed early onset and severe symptoms, the father had mild symptoms, which were different from his children (**Figure 1B**, II-5). He had mild distal limb weakness in his thirties, which resulted in gait disturbance. The symptoms were slowly progressive and he had difficulty in climbing stairs. There were no deformities in his hands and feet. Gastrocnemius muscles atrophy was observed in the lower extremities. Neurological examination revealed his foot plantar flexion and dorsal flexion were grade 4/5. He had mild sensory loss of feet. Tendon reflexes were sluggish. The electromyography results are shown in **Table 2**. His CMTNS was 7.

Histopathological findings

The sural nerve biopsy was performed in the II-5. Electron microscopic assessment of semi-

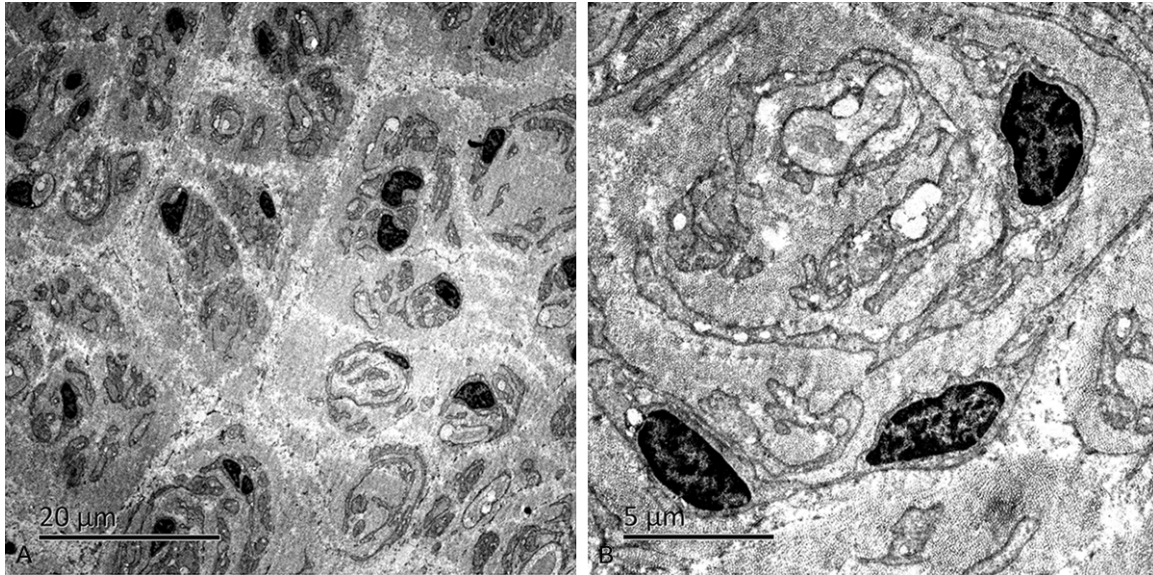


Figure 2. Histopathological findings of the Sural Nerve Biopsy in the affected family member (II-5). Electron micrograph. Transverse section. A. There is a significant decrease of myelinated fibers, residual fibers have abnormally thin myelin sheaths. B. A typical onion bulb.

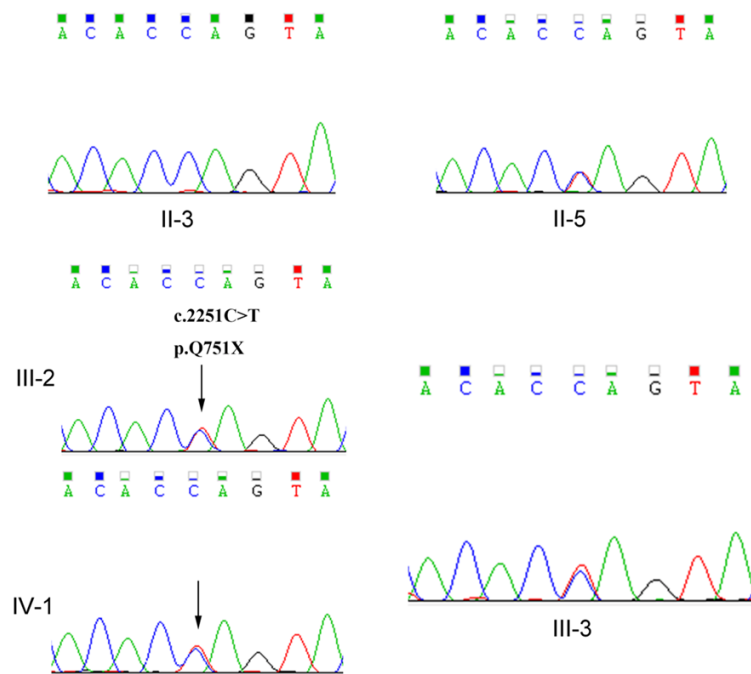


Figure 3. Mutation in *MFN2* gene. A heterozygous change of c.2251C>T (p.Q751X) in exon 19 of *MFN2* was identified in proband, II-5, III-3 and IV-1; II-3 is normal controls.

thin resin sections showed that the myelinated fibers were remarkably decreased, especially large-sized fibers. The residual fibers had abnormally thin myelin sheaths (**Figure 2A**). Typical onion bulb around an abnormally myelinated fiber was seen (**Figure 2B**).

Genetic findings

We performed a targeted multi-gene panel screening, containing 64 disease-causing genes of CMT [Supplementary Data](#) and related disorders, for the proband. We detected known pathogenic mutation with heterozygous change: a nonsense mutation in *MFN2* (c.2251C>T, p.Q751X) [5] (**Figure 3**). The nonsense mutation resulted in a premature stop codon in the final exon of the gene, which caused CMT2A. The mutation segregated with the disease in this family and was absent in the 1,000 Genomes project database and Exome Aggregation Consortium. We performed prenatal diagnosis and confirmed that her fetus also carried this mutation.

Discussion

Mutations in *MFN2* gene cause 20% of CMT2 cases [11]. *MFN2* is mapped to chromosome 1p36.22, and contains 19 exons. The gene *MFN2* encodes Mitofusin-2 integral outer mitochondrial membrane protein that participates in modulating mitochondrial morphology by

promoting mitochondrial tethering and fusion [12]. More than 100 different *MFN2* mutations have been reported till date.

In this family, we identified a nonsense mutation Q751X in the *MFN2* gene. The same mutation has been previously reported [5]. This mutation is predicted to generate a truncated protein. The mRNA containing a nonsense mutation is highly unstable because it can be degraded by nonsense-mediated mRNA decay (NMD) [13]. Therefore, in patients with this nonsense mutation, there may be complete loss of the Mitofusin-2 protein, thereby affecting mitochondrial tethering and fusion.

CMT2A associated with *MFN2* mutations is clinically heterogeneous, ranging from mild to severe forms. Generally, patients with an early onset are associated with severe phenotype, while those with a late onset show more benign clinical course. The mutation Q751X was reported to be only associated with an early onset and severe phenotype of CMT2A [7], but there was no further clinical evaluation of that family [7]. In this family, we found that the proband and her brother had early onset and severe phenotypes (CMTNS \geq 21), but the father showed late onset with slow progressive course and mild phenotype (CMTNS \leq 10). This inter-familial phenotypic variability caused by the same mutation is unique. It is unclear whether this inter-familial phenotypic variability may be due to environmental or other factors that modulate genetics [14].

In summary, the *MFN2* Q751X mutation was responsible for CMT2A in a Chinese Han family. Our results expand the clinical features of CMT2A caused by *MFN2* Q751X mutation. We conducted prenatal diagnosis of the proband's fetus to provide scientific guidance and promote early intervention for CMT patients, which may minimize the harmful effects of the disease.

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Disclosure of conflict of interest

None.

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