



*Case Report*

## **A novel mutation of the MFN2 gene in a Chinese family with Charcot-Marie-Tooth disease**

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**ABSTRACT.** Charcot-Marie-Tooth (CMT) is a group of clinically and genetically heterogeneous inherited neuromuscular disorders. At present, more than 30 loci have been reported to be associated with CMT disease; point mutations in the mitofusin 2 (MFN2) gene is one of the most common causes. We studied a Chinese family with CMT disease in which the phenotype of affected individuals varied, and the weakness condition of the distal legs in males, except the proband, was less severe than in females in this family. Linkage analysis and PCR sequencing revealed a missense mutation (NM\_014874.3:c.1066 A>G) in the MFN2 gene, resulting in an amino acid substitution of threonine to alanine in condon 356 (Thr356Ala). This is a novel phenotype and mutation for CMT family.

**Key words:** Charcot-Marie-Tooth; Mitofusin 2; Mutation; Gender

## INTRODUCTION

Charcot-Marie-Tooth (CMT) disease is a genetically and clinically heterogeneous group of inherited peripheral neuropathies, with a prevalence of 17-40 per 100,000 individuals (Barisic et al., 2008). The clinical features are a variable age of onset of progressive distal muscle weakness and atrophy, starting in the lower limbs and subsequently affecting the upper extremities (Nicolaou et al., 2010). Point mutations in the mitofusin 2 (MFN2) gene are some of the most common causes of CMT. In 2004, the MFN2 gene was reported to be associated with CMT by means of linkage analysis (Züchner et al., 2004), and since then, about 60 point mutations in the MFN2 gene have been described (Cartoni and Martinou, 2009).

In this study, we identified a novel missense mutation (NM\_014874.3:c.1066 A>G, p.Thr356Ala) in the MFN2 gene in a Chinese family with CMT disease. We also characterized the phenotype of this family, which differed from that in other reports.

## MATERIAL AND METHODS

### The proband

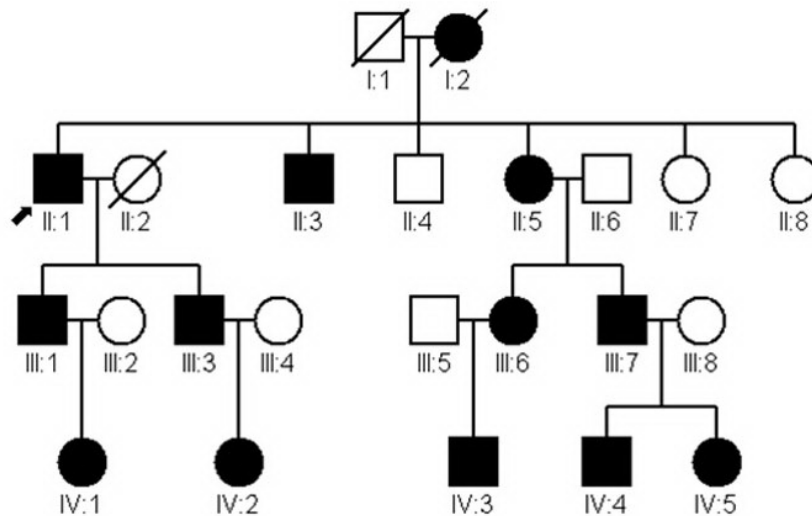
The proband was a 65-year-old man who had hollow foot, claw hand deformity, and severe muscle atrophy of the lower and upper distal extremities. Deep tendon reflexes of lower distal extremities were absent and sensory disturbances were mild. He was the worst one in this family and currently wheelchair-bound (Figure 1). His age onset was about 8 years. Neurophysiological data showed that motor nerve conduction velocity (MCV) was 37.4 m/s, the sense nerve conduction velocity (NCV) was slightly reduced, the compound nerve action potential (CNAP) of the right common peroneal was also reduced, and the CNAP of the left common peroneal and the compound motor action potential (CMAP) of the bilateralis could not be elicited.



**Figure 1.** The phenotype of the proband. The proband had hollow foot (A), claw hand deformity and severe muscle atrophy of lower and upper distal extremities (B, C).

## The family

His family was a four-generation group with 20 members including 12 symptomatic cases, the ages at disease onset ranged from 6 to 50 years and the disease status varied (Figure 2). The symptoms of all 5 females in this family were distinct. One girl ( $\chi$ :2) showed steppage gait, pes cavus, difficulty in walking because of distal muscle weakness in legs. She fell down frequently since 6 years old and had a quick progression of disease. Another girl ( $\chi$ :5) was an undergraduate student who showed mild steppage gait, muscle weakness in distal legs and upper arms. She persevered with physical exercise for years but was unable to run. Her age of disease onset was 11 years. The three other females showed symptoms of pes cavus, muscle weakness in lower legs or upper arms. The disease status of males in this family excluding the proband was slight, only mild muscle weakness could be detected and their action potential was normal.



**Figure 2.** The pedigree structure of the family. The pedigree structure showing a four-generation family with 20 members including 12 affected individuals.

## Linkage analysis

After informed consent and approval by the local Ethics Committee, 20 blood samples from these family members, including 12 affected individuals, were obtained. Genomic DNA was extracted from peripheral blood leukocytes using the standard phenol/chloroform method. Based on the UCSC Genome Browser on Human 2009 Feb assembly (<http://www.genome.ucsc.edu>), 6 short tandem repeats (STRs) close to the MFN2 gene (D1S503, D1S224, D1S2667, D1S489, D1S434, and D1S228) were selected as genetic markers for two point linkage analysis. The sequences of primers were from the STR information in UCSC. PCR was performed under standard conditions. The PCR products were separated by electrophoresis on 8% denaturing polyacrylamide gels, and allele fragments were detected with routine silver staining. Two-point linkage analysis was carried out using the MLINK program of the LINKAGE Package (version 5.2).

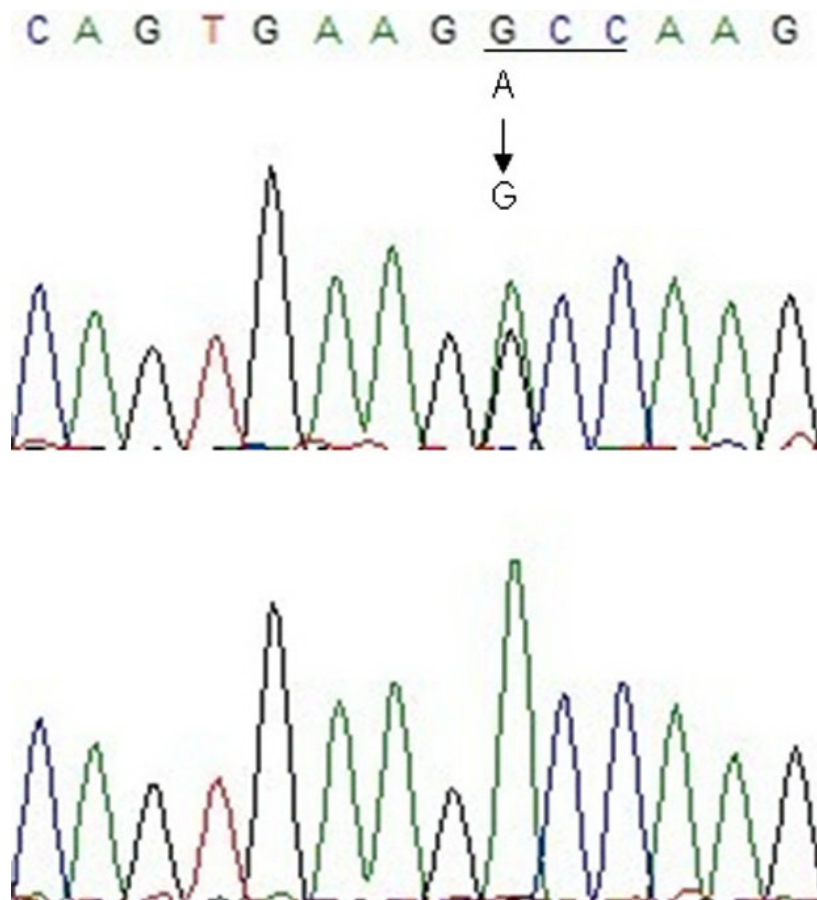
### Mutation analysis

Mutations were detected by direct sequencing of the PCR products flanking all the exons, intron-exon boundaries and the 5'- and 3'-flanking regions of the MFN2 gene in both directions. An ABI 3730 automatic sequencer was used.

### RESULTS

Two-point linkage analysis generated 2 positive LOD scores of 3.61 and 2.03 with the marker D1S489 and D1S2667, respectively, showing definitive evidence of linkage.

Sequence analysis identified a heterozygous mutation in cDNA1066 A>G (NM\_014874.3), leading to a missense substitution in codon 356 from threonine to alanine (Thr356Ala) (Figure 3). No mutation was found in other family members (excluding 12 patients) and 200 control individuals by restriction digest with HpyCH4IV.



**Figure 3.** Sequencing analysis of MFN2 exon 11 (the proband). Direct sequencing showing the position of the heterozygous A-to-G substitution (c.1066 A>G), resulting in the mutation Thr356Ala.

## DISCUSSION

The phenotype of CMT disease varies especially in families, which can range from severe muscle atrophy to no symptoms in affected individuals. Some studies have reported that the phenotype is markedly different in early (<10 years) and late disease-onset (>10 years) CMT groups with MFN2 mutations, where patients with early onset usually involve severe cases and most patients with late onset show mild symptoms (Chung et al., 2006; Banchs et al., 2008). The same phenomenon occurred in this family, where 3 patients with severe disease status had early onset (the proband,  $\chi$ :2 and  $\chi$ :5) at 8, 6 and 11 years, respectively; they had malformation in extremities and severe muscle atrophy and were even wheelchair-bound. The onset ages of the other 9 patients were all later than 16 years and showed milder muscle weakness in lower legs or upper arms. Moreover, there was an interesting detection that the weakness condition of the distal legs in males was less than in females, except for the proband in this family. This has not been mentioned by any reported CMT. Further investigations uncovered that all the males except the proband in this family were manual laborers; they were active for hours everyday but the females were not. This situation led us to make a bold conjecture that the subjective movement may be helpful in relieving the objective muscle weakness in CMT patients, but it is only a hypothesis and needs more studies to verify it.

MFN2 is a mitochondrial GTPase protein that regulates the mitochondrial network architecture by fusion of mitochondria, it contains a GTPase domain near the N-terminus, a coiled-coil domain, two transmembrane spans and a coiled-coil domain in the C-terminal tail (Rojo et al., 2002; Verhoeven et al., 2006). All the mutations in the MFN2 gene were predicted to affect each region of the protein, but most had been detected for the GTPase domain and the region linking the GTPase domain and the first coiled-coil region, they were two hotspot regions of reported MFN2 mutations in CMT disease (Engelfried et al., 2006; Cartoni and Martinou, 2009). In this family, we revealed a novel substitution mutation in aa356 (Thr356Ala), which also fell in the hotspot region linking the GTPase domain and the first coiled-coil region, and was immediately next to the mutation (Lys357Asn) reported in Japanese patients (Kijima et al., 2005). It indicates that MFN2 mutations occurring in the Chinese population may be similar with other ethnic groups, and the hotspot regions in MFN2 function are important.

Functional coiled-coil regions are essential for tethering of mitochondria before fusion (Koshiha et al., 2004), and an intact GTPase domain is indispensable for the function of mitofusins (Santel and Fuller, 2001). There are two highly conserved regions between the GTPase domain and the first coiled-coil region, which are the important segments of the protein that could provide binding sites for the assembly (Honda et al., 2005). The mutation in our study located in the region immediately upstream of the first coiled-coil region (Thr356Ala), possibly changes the linking structure of the GTPase domain and the coiled-coil region, which may affect the function of the two regions, and the binding sites for the assembly in the two highly conserved regions may also be destroyed.

In conclusion, we described a novel missense mutation in the MFN2 gene (NM\_014874.3:c.1066 A>G, p. Thr356Ala) in a Chinese family with CMT disease. The mutation was located in a hotspot region of reported MFN2 mutations. The disease status was different in patients with different age onsets and genders, which has not been previously reported especially for gender.

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