A novel TFG c.793C>G mutation in a Chinese pedigree with Charcot-Marie-Tooth disease 2

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Abstract

Introduction: Mutations within TFG gene were recently reported to cause Charcot-Marie-Tooth disease 2 (CMT2). However, only few pedigrees were documented so far. Here, we reported a Chinese CMT2 pedigree with 8 affected cases and a novel TFG mutation.

Methods: Clinical evaluation and electrophysiological study were performed in all the affected individuals. Whole-exome sequencing was conducted, followed by the Sanger sequencing and co-segregation analysis to verify the variants.

Results: All cases presented with a phenotype of CMT2, including slowly progressive symmetrical muscle atrophy and weakness predominantly in the distal limbs. Sensory loss in the distal limbs was present in the proband and his father. Age at onset ranged from 37 to 44 years, and was younger in male cases, compared with female cases. Nerve conduction study revealed normal motor nerve conduction velocity but decreased compound muscle action potential. Electromyography test revealed fibrillation potential and positive sharp waves. The creatine kinase activity was increased in all cases. After genetic investigations, we identified a novel TFG c.793C>G (p.Pro265Ala) mutation in the family. This mutation alters the conserved amino acid residue and is absent in 1000G, ExAC, dbSNP, EP6500, and 200 in-house controls. It co-segregated with the disease in the family.

Conclusions: Our report provided additional evidence that the heterozygous TFG mutations were associated with CMT2.

KEYWORDS
Charcot-Marie-Tooth disease 2, distal muscle atrophy, motor nerve conduction velocity, novel mutation, TFG

Ding-Wen Wu and Yanfang Li contributed equally to this work.

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1 | INTRODUCTION

Charcot-Marie-Tooth disease (CMT) is the most common form of inherited neuropathies with great clinical and genetic heterogeneity (Rossor, Polke, Houlden, & Reilly, 2013). The prevalence of CMT is estimated at 17-40/100000 (Barreto, Oliveira, & Nunes, 2016). According to the nerve conduction velocity (NCV) of median motor, CMT is divided into three types: demyelinating (CMT1) with NCV < 35 m/s, axonal (CMT2) with NCV > 45 m/s, and intermediate CMT (ICMT) with NCV 35–45 m/s (Mathis, Goizet, & Tazir, 2015; Thomas, Guergueltcheva, & Gondim, 2016). Patients with CMT usually suffer from slowly progressive muscle weakness and atrophy of distal extremities and sensory loss of distal end. The symptoms usually begin in the first decade to the third decade but can be great variable. The inherited pattern of CMT includes autosomal dominant, autosomal recessive, and X-linked. To date, more than 80 genes have been associated with CMT (Pareyson, Saveri, & Pisciotta, 2017). Among these identified genes, PMP22 duplication is the most common cause, accounting for 40%–50% of all CMT and about 70% of 8 patients were involved in this family. Nerve conduction study and electromyography (EMG) were performed in available individuals. PMP22 duplication/deletion was excluded before the whole-exome sequencing (WES). Besides, 200 normal individuals with no history of neurological disorders were collected as controls.

2.2 | Genetic investigations

Genomic DNA was extracted from peripheral blood, using QIAamp genomic DNA kits (Qiagen). WES was performed in the proband, followed by the Sanger sequencing to verify the variant. Co-segregation analysis was conducted in all available familial members. Minor allele frequency (MAF) was searched on ExAC, dbSNP, and 1,000 Genomes Browser. In silico algorithms SIFT, PolyPhen-2, Mutation Taster, and CADD were used for functional prediction analysis. Variants were classified according to American College of Medical Genetics and Genomics (ACMG) guideline.

3 | RESULTS

3.1 | Clinical features

A total of 8 affected individuals were included in this four-generation pedigree (Figure 1a). The inheritance pattern was autosomal dominant. Age at onset ranged from 37 to 44 years. All affected individuals presented with CMT2 phenotypes, including slowly progressive symmetrical muscle atrophy and weakness predominantly in the distal limbs. Sensory loss in the distal limbs was present in the proband and his father. No sensory impairment was detected in other affected individuals. The median motor NCV ranged from 54.6 to 62.1 m/s (Table 1). Compound muscle action potential (cMAP) was decreased. EMG test revealed fibrillation potential and positive sharp waves in the proband and his older sister (III-12). The creatine kinase (CK) was increased in all cases.

The proband (III-14) was a 44-year-old man who had a 6-year history of muscle weakness and atrophy in four extremities. At the age of 38, he felt muscle weakness in his lower limbs after exercise. The weakness slowly progressed in the following months. Half a year after the onset, he noticed muscle atrophy at the distal end of lower limbs. There was no sensory dysfunction at early stage. At the age of 41, he exhibited muscle atrophy in his hands and his fine movement was impaired. In addition, he presented with muscle cramps in his four extremities. Neurological examinations at the age of 44 revealed obvious muscle atrophy in bilateral intesosseus muscle and gastrocnemius muscle (Figure 1b, b1-b2). Fasciculation could be observed in his lower limbs. Muscle strength (Medical Research Council—MRC scale) showed 4/5 for distal upper limbs, 3/5 for distal lower limbs, and 5/5 for proximal limbs. Deep tendon reflexes were absent in the lower limbs and decreased in the upper limbs. Plantar responses were flexor. Cranial examination, cerebellar, and autonomic functions were unremarkable. Tactile and pain sensations were impaired in the distal limbs. Vibratory sensation was...
normal. Laboratory tests revealed normal blood cell count, liver function, renal function, serum glucose, and low-density lipoprotein. However, CK was increased, 637 U/L (normal < 171 U/L). The NCV was 62.1 m/s in the median nerve and 46.4 m/s in the peroneal nerve. The cMAP was 0.922 mV in the median nerve and 1.2 μV in the sural nerve. EMG revealed profuse fibrillation potentials and positive sharp waves with long-duration, high-amplitude, polyphasic muscle unit action potentials.

The father (II-6), aged 76 at present, exhibited muscle atrophy at the age of 38. The amyotrophy commenced at the distal end of upper limbs and gradually developed to the lower limbs. Muscle cramps of four extremities were obvious at the early stage of the disease. Also, he complained about pain of fingers in the cold water. At the age of 71 years, he lost ambulation and became bedridden. Neurological examinations revealed extensive muscular atrophy, especially in deltoid, triceps, forearm, interosseous, tibialis anterior, and gastrocnemius muscle (Figure 1b, b3-b4). Muscle strength revealed 3/5 in the proximal limbs, 1/5 in the distal upper limbs, and 0/5 in the distal lower limbs. Touch and pain sensations were decreased in hands and feet. Vibratory sensation was slightly decreased in ankle joints. Deep tendon reflex was disappeared in all limbs. Plantar responses were flexor. EMG test and laboratory examinations were refused.

The 51-year-old sister (III-10) suffered from muscle atrophy of distal upper limbs at the age of 43. Her amyotrophy was predominant in interosseous, thenar, and gastrocnemius muscle (Figure 1b, b5-b6). She also complained of recurrent muscle cramps. Neurological examinations revealed muscle strength 3/5 in the distal limbs and 5/5 in the proximal limbs. Knee reflex was normal, while ankle reflex was disappeared. Sensory examinations were negative. Plantar responses were flexor. Another sister (III-12) had initial symptoms of muscle weakness and cramps in the lower limbs at the age of 38. Several months later, she noticed muscle atrophy in her hands. Neurological examinations revealed muscle atrophy in bilateral interosseous muscle, 4/5 muscle strength in the distal upper limbs. Fasciculation was apparent in her lower limbs. There was no sensory impairment in all limbs. CK was increased, 358 U/L (normal < 171 U/L). The NCV revealed decreased cMAP of peroneal nerve and tibial nerve. EMG revealed fibrillation potentials with high-amplitude, polyphasic muscle unit action potentials. His 45-year-old cousin (III-8) had an age at onset of 38 years. The initial symptoms included weakness of hands and atrophy of interosseous muscle. Muscle cramps were also complained. Muscle atrophy was seen in interosseous muscle, thenar muscle, and short peroneal (Figure 1b, b7-b8). Muscle strength was 3/5 in the distal upper limbs and 4/5 in the distal lower limbs. Sensory examination was unremarkable. Another cousin (III-6), aged 49, exhibited muscle atrophy of limbs at the age of 45. Her muscle strength was 4/5 in the distal upper limbs and 5/5 in
remaining muscles. No sensory loss was present in her limbs. CK was 247 U/L (normal < 171 U/L). NCV revealed decreased cMAP of peroneal nerve and tibial nerve.

Individual III-7 (aged 47) had no symptom of muscle atrophy and weakness. His EMG test was unremarkable, with normal motor NCV (61.1 m/s in the median nerve, 46.0 m/s in the peroneal nerve) and normal cMAP (9.8 mV in the median nerve, 10.1 mV in the peroneal nerve). The SNAP was 10.3 and 17.1 μV in the median nerve and sural nerve, respectively. CK was within the normal level (66 U/L). The other familial members II-1, III-1, III-2, III-3, III-4, and III-5 did not exhibit muscle weakness or atrophy at present. II-2 had passed away and was reported not to have muscle atrophy. NCV study and EMG test were not performed in these members.

3.2 | Genetic analysis

Using WES, we identified a heterozygous TFG (NM_001195478) mutation c.793C>G in the proband (Figure 1c). This mutation causes a substitution of proline 265 with alanine (p.Pro265Ala) and resides in an evolutionarily conserved region (Figure 1d). This mutation was also detected in II-6, III-6, III-8, III-10, and III-12, all of whom were affected individuals. We did not find this mutation in II-1, III-1, III-2, III-3, III-4, III-5, and III-7. Co-segregation with the disease was confirmed in the family. This mutation was located in the conserved amino acid residue and was absent in 1000G, ExAC, dbSNP, EP6500, and our 200 in-house controls. It is predicted to be tolerable by SIFT, benign by PolyPhen-2, disease causing by Mutation Taster, and damaging by CADD. According to the ACMG guideline, this mutation should be classified as "likely pathogenic."

4 | DISCUSSION

Here, we presented a novel TFG mutation in a Chinese CMT2 pedigree. The mutation p.Pro265Ala alters the conserved amino acid residue and is absent in public genomic database and our in-house controls. In addition, the location of this mutation was adjacent to the pathogenic mutation p.Gly269Val. The co-segregation in the family revealed that this mutation might be causative for the disease. Due to the technical and material limitations, the evaluation of functional alternation of the mutant protein was not conducted. Since loss-of-function mechanism has been reported as molecular pathogenesis of TFG, we believed this novel mutation may be biologically pathogenic.

The patients in this pedigree exhibited symmetrical muscle atrophy and weakness predominantly in the distal limbs. Distal sensory impairment was present in the proband and his father. Motor nerve conduction studies were within normal limits. EMG showed denervation and reinnervation. Based on the clinical and electrophysiologic findings, these patients met the diagnosis criteria of CMT2. In some patients, sensory impairment was not present. It is possible that their sensory loss was mild, similar to the CMT2 patients with TFG p.Gly269Val mutation (Tsai et al., 2014). Interestingly, some patients in our study had onset of muscle atrophy in the upper limbs, which is consistent with the patients described by Fabrizi, Høyer,

### TABLE 1

|                | II-6 | III-6 | III-8 | III-10 | III-12 | III-14 |
|----------------|------|-------|-------|--------|--------|--------|
| Gender         | M    | F     | M     | F      | F      | M      |
| Age at onset   | 38   | 44    | 37    | 43     | 41     | 38     |
| Age at study   | 76   | 49    | 45    | 51     | 48     | 44     |
| Strength, thumb abduction (MRC) | 1    | 4     | 3     | 3      | 4      | 4      |
| Strength, foot dorsiflexion (MRC) | 0    | 5     | 4     | 4      | 5      | 3      |
| Distal muscle atrophy UL | Marked | Mild | Marked | Marked | Mild | Marked |
| Distal muscle atrophy LL | Marked | Mild | Marked | Moderate | Normal | Marked |
| Sensory loss   | Distal | Normal | Normal | Normal | Normal | Normal |
| Knee reflex    | Areflexia | +     | ++    | ++     | Areflexia | Areflexia |
| Ankle reflex   | Areflexia | Areflexia | Areflexia | Areflexia | Areflexia | Areflexia |
| Median nerve MNCV (m/s) | NA | 54.6 | NA | NA | 56.5 | 62.1 |
| Median nerve cMAP (mV) | NA | 14.4 | NA | NA | 11.4 | 0.922 |
| Peroneal nerve MNCV (m/s) | NA | 52.6 | NA | NA | 42.6 | 46.4 |
| Peroneal nerve cMAP (mV) | NA | 4.3 | NA | NA | 2.08 | 2.4 |
| Median nerve SNAP (μV) | NA | 52.0 | NA | NA | 50.4 | 49.1 |
| Sural nerve SNAP (μV) | NA | 26.8 | NA | NA | 8.9 | 1.2 |

Abbreviations: cMAP, compound muscle action potential; LL, lower limbs; MNCV, motor nerve conduction velocity; MRC, Medical Research Council scale; NA, not available; SNAP, sensory nerve action potential; UL, upper limbs.
CONFLICT OF INTEREST
The authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS
Ding-Wen Wu contributed to funding, data acquisition, analysis and interpretation, and drafting of the manuscript. Yanfang Li contributed to data acquisition, analysis and interpretation, drafting of the manuscript. Xinzhen Yin contributed to data acquisition. Baorong Zhang contributed to study design, study supervision, data acquisition, analysis and interpretation of data, and critical revision of the manuscript.

DATA AVAILABILITY STATEMENT
The data supporting the results of this study are publicly available.

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