Genetic profile of Chinese patients with Charcot-Marie-Tooth disease

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To the Editor: Charcot-Marie-Tooth disease (CMT) encompasses a genetically heterogeneous group of inherited neuropathies, characterized by progressive distal muscle weakness and atrophy, sensory deficits, impaired tendon reflexes, and foot deformities.[1] To date, more than 80 causative genes have been identified in CMT patients, associated with either autosomal dominant or recessive inheritance, or X-linked transmission. The traditional classification of CMT was based on peripheral neuropathy type, as determined by nerve conduction velocity. As more causative genes were identified and the overlap of neuropathy phenotypes became apparent, the traditional classification system proved unwieldy and inadequate. Moreover, CMT needs to be distinguished from several entities including systemic disorders with neuropathy and other types of hereditary neuropathy. In clinical practice, overlap of phenotypes can present a major challenge in reaching the correct diagnosis. This study aimed to investigate the genetic profile in a cohort of Chinese CMT patients and evaluate the role of genetic testing in the diagnosis and subtyping of CMT.

A total of 66 unrelated Chinese probands with CMT were enrolled from March 2004 to April 2019. Neurological examinations were performed by two experienced neurologists. For 36 cases collected before May 2013, three microsatellite markers within the 1.4 Mb duplication region on chromosome 17p11.2-p12 were first used to detect *PMP22* duplication. In probands with no *PMP22* duplication identified, mutational analysis of *PMP22*, *GJB1*, and *MPZ* genes was further performed using denaturing high-performance liquid chromatography (DHPLC). For 30 cases collected after May 2013, multiplex ligation-dependent probe amplification was first performed to detect *PMP22* duplication. In probands with negative results, next-generation sequencing, either whole-exome sequencing ($n = 1$) or use of a targeted sequencing panel ($n = 5$), was carried out to identify causative genes. Candidate mutations found by either DHPLC or next-generation sequencing were further confirmed by Sanger sequencing.

The most common cause of CMT in our cohort was *PMP22* duplication (28/66, 42.4%). Four missense, c.22A>T (p.T8S), c.65G>T (p.R22L), c.223C>T (p.R75W), c.392T>C (p.L131P), and one nonsense mutations, c.64C>T (p.R22*), were identified in the second most common causative gene *GJB1* (5/66, 7.6%). The novel variant c.22A>T (p.T8S) was not found in the population databases including 1000 Genomes Project and the Exome Aggregation Consortium. Co-segregation analysis was not performed because family members' DNA samples were not available. Two known causative mutations, c.22A>C (p.T8P) and c.23C>T (p.T8I), have been found in codon 8, suggesting the critical biological function of this conserved residue. The other novel variant, c.65G>T (p.R22L) was also not found in the population databases mentioned above. This variant was detected in the proband and his affected mother. Four known causative mutations, c.64C>G (p.R22G), c.64C>T (p.R22*), c.65G>A (p.R22Q), and c.65G>C (p.R22P), have been found in codon 22, strongly indicating that this position was highly conserved through evolution. The American College of Medical Genetics and Genomics (ACMG) classification of both variants was "likely pathogenic."

A novel homozygous *FGD4* missense variant c.1303C>T (p.R435W) was identified in a 38-year-old woman born to

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Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001095

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Chinese Medical Journal 2020;133(21)
Received: 05-07-2020 **Edited by:** Xin Chen

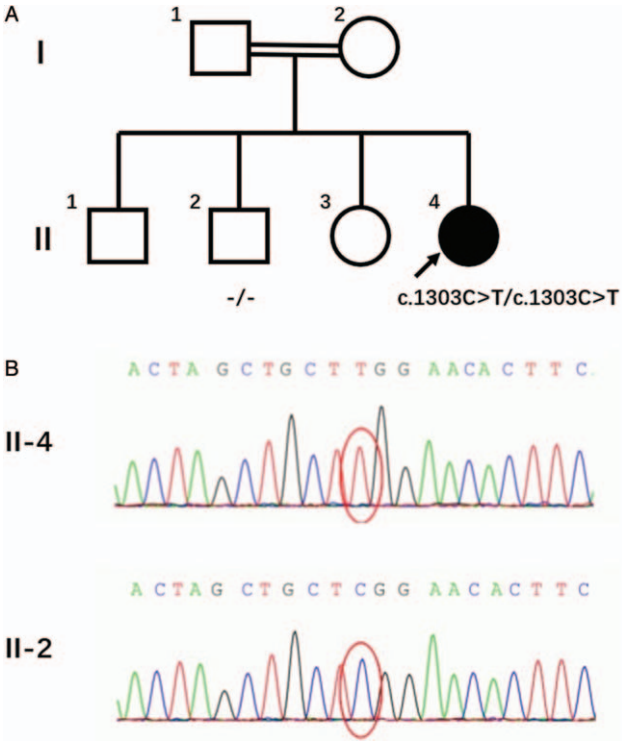

Figure 1: (A) Family pedigree. The proband (II-4) harbored a homozygous mutation c.1303C>T in the *FGD4* gene. Her unaffected brother (II-2) did not carry the mutation. (B) Sequence chromatograms of the proband and her unaffected brother.

a consanguineous family [Figure 1]. This mutation was not found in the population databases and was predicted to be pathogenic by most *in-silico* tools (http://varcards.biols.ac.cn). The patient first noticed pes cavus when she was 8 years old. She developed facial palsy at the age of 28 and limb numbness at the age of 37. Electromyography revealed peripheral neuropathies (predominantly demyelinating neuropathies) and facial nerve impairment. The variant could be classified as "likely pathogenic" on the basis of ACMG guidelines.

A novel heterozygous *FIG4* variant c.923C>T (p.A308V) was found in a 22-year-old woman and her affected father. The variant was found in the population databases with a frequency lower than 0.01 and predicted to be disease-causing by multiple in-silico tools. The proband had suffered from left hand numbness 3 years previously and subsequently developed numbness in all four limbs, mainly affecting the left side. Electromyography revealed left-sided ulnar nerve impairment. Her father had suffered from left hand numbness at the age of 46. Electromyography demonstrated bilateral ulnar nerve deficits.

Our study identified mutations in common causative genes (eg, *PMP22* and *GJB1*) and rare ones (eg, *FGD4* and *FIG4*), further expanding the mutational spectrum of CMT-related genes. CMT4H is a rare autosomal recessive hereditary neuropathy caused by *FGD4* mutations and characterized by first-decade onset, slowly progressive distal muscle weakness, frequent scoliosis, and myelin outfoldings visible in nerve biopsy samples.[2] Cranial nerve involvement was only reported in one case, a 65-year-old man who had apparent external ophthalmoplegia, facial muscle palsies, and bilateral inner ear hearing loss.[3] Our case further supported cranial nerve involvement as a rare presentation in patients with CMT4H.

Biallelic *FIG4* mutations are the cause of CMT4J while heterozygous mutations are associated with amyotrophic lateral sclerosis 11 (ALS11).[4,5] CMT4J is a peripheral neuropathy characterized by childhood onset with accelerated limb weakness and muscle atrophy during the teenage years or adulthood that involve both distal and proximal limb muscles, while ALS11 had a less severe phenotype with adult onset and prominent corticospinal tract findings. The clinical significance of the heterozygous mutation c.923C>T found in both the proband and her affected father with ulnar nerve deficits remains unclear. More case reports are needed to demonstrate whether this could present a new phenotype of *FIG4*-related disorders.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Funding

The study was supported by grants from the Science and Technology Department of Zhejiang Province (No. 2019C03017) and the National Natural Science Foundation of China (No. 30672264, No. 81870895, No. 81600850, No. 81571089, and No. 81371266).

Conflicts of interest

None.

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How to cite this article: Ouyang ZY, Chen Y, Qin DQ, Cen ZD, Zheng XS, Xie F, Chen S, Wang HT, Yang DH, Chen XH, Wang LB, Zhang BR, Luo W. Genetic profile of Chinese patients with Charcot-Marie-Tooth disease. Chin Med J 2020;133:2633–2634. doi: 10.1097/CM9.0000000000001095