Screening for SH3TC2, PMP2, and BSCL2 Variants in a Cohort of Chinese Patients with Charcot-Marie-Tooth

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Abstract

Background: SH3TC2, PMP2, and BSCL2 genes are related to autosomal recessive (AR) Charcot-Marie-Tooth (CMT) disease type 1, autosomal dominant (AD)-CMT1, and AD-CMT2, respectively. Pathogenic variants in these three genes were not well documented in Chinese CMT patients. Therefore, this study aims to detect SH3TC2, PMP2, and BSCL2 pathogenic variants in a cohort of 315 unrelated Chinese CMT families.

Methods: A total of 315 probands from 315 unrelated Chinese CMT families were recruited from the Department of Neurology of Third Xiangya Hospital and Xiangya Hospital. We screened for SH3TC2 pathogenic variants in 84 AR or sporadic CMT probands, PMP2 pathogenic variants in 39 AD or sporadic CMT probands, and BSCL2 pathogenic variants in 50 AD or sporadic CMT2 probands, using polymerase chain reaction and Sanger sequencing. All these patients were out of 315 unrelated Chinese CMT families and genetically undiagnosed after exclusion of pathogenic variants of PMP22, MFN2, MPZ, GJB1, GDAP1, HSPB1, HSPB8, EGR2, NEFL, and RAB7. Candidate variants were analyzed based on the standards and guidelines of American College of Medical Genetics and Genomics (ACMG). Clinical features were reevaluated.

Results: We identified three novel heterozygous variants such as p.L95V (c.283C>G), p.L1048P (c.3143T>C), and p.V1105M (c.3313G>A) of SH3TC2 gene and no pathogenic variants of PMP2 and BSCL2 genes. Although evaluation in silico and screening in the healthy control revealed that the three SH3TC2 variants were likely pathogenic, no second allele variants were discovered. According to the standards and guidelines of ACMG, the heterozygous SH3TC2 variants such as p.L95V, p.L1048P, and p.V1105M were considered to be of uncertain significance.

Conclusions: SH3TC2, PMP2, and BSCL2 pathogenic variants might be rare in Chinese CMT patients. Further studies to confirm our findings are needed.

Key words: BSCL2; Charcot-Marie-Tooth Disease; PMP2; SH3TC2

INTRODUCTION

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous group of hereditary peripheral neuropathies with an estimated prevalence of 1:2500.[1] It is characterized by distal muscle weakness and atrophy, distal sensory loss, areflexia, and pes cavus.[1] According to electrophysiological features, CMT is divided into CMT1 (median nerve conduction velocity [MNCV] <38 m/s) and CMT2 (MNCV >38 m/s).[2] To date, more than eighty genes have been identified to be associated with CMT (http://neuromuscular.wustl.edu/time/hmsn.html).

SH3TC2 pathogenic variants were first identified to cause autosomal recessive (AR)-CMT1 in 12 Germany families by Senderek et al. and were mainly distributed in Mediterranean families.[3] SH3TC2-associated CMT is an AR demyelinating neuropathy generally characterized by a slower MNCV and more severe clinical phenotype than CMT1A.[4] The gene SH3TC2 encodes for the SH3 domain containing 2 protein, which is involved in the regulation of various axonal growth and function, and the gene is expressed in peripheral nerves.[5] A few reports have documented the significance of SH3TC2 in CMT,[6] and the new creations are licensed under the identical terms. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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Peripheral neuropathy with scoliosis and cranial nerve disturbances being its prominent clinical features.[4] PMP2 pathogenic variants were recently identified to cause autosomal dominant (AD)-CMT1.[5] To date, a total of three pathogenic variants have been reported worldwide and no Chinese families have been reported.[6,7] The clinical features of TOMP2-associated neuropathy were similar to PMP2 duplication.[6,8] BSCL2 pathogenic variants were originally identified in patients with AR congenital generalized lipodystrophy type 2 and were subsequently found to cause a broad spectrum of neurological disorders.[9] The BSCL2 pathogenic variant p.S90W was identified to cause AD-CMT2 in a Korean family, who presented predominant thenar muscle atrophy, frequent sensory disturbances, and pyramidal tract signs.[10]

Since pathogenic variants of SH3TC2, PMP2, and BSCL2 were not well documented in Chinese CMT patients, this study aims to screen for pathogenic variants of these three genes in a cohort of Chinese CMT patients and describe the clinical features of patients carrying pathogenic variants.

**Methods**

**Ethical approval**

This study was approved by the Ethics Committee of the Third Xiangya Hospital and Xiangya Hospital. Informed consent was obtained from all the participants.

**Patients**

A total of 315 probands from 315 unrelated CMT families were recruited from the Department of Neurology of the Third Xiangya Hospital and Xiangya Hospital (including 140 CMT1 families, 158 CMT2 families, and 17 unclassified families). Of the 315 CMT families, 96 were AD-CMT, 40 were X-linked CMT, 134 were sporadic CMT, and 21 were unclassified. Eighty-four genetically undiagnosed probands with AR or sporadic CMT were screened for SH3TC2. Thirty-nine genetically undiagnosed probands with AD or sporadic CMT1 were screened for PMP2. Fifty genetically undiagnosed probands with AD or sporadic CMT2 were screened for BSCL2. These patients were negative for pathogenic variants of PMP22, MFN2, MPZ, GJB1, GDAP1, HSPB1, HSPB8, EGR2, NEFL, and RAB7. All the index patients were diagnosed as CMT according to the 2nd Workshop of the European CMT Consortium.[11] For each variant, 100 Chinese healthy individuals were, respectively, recruited into this study. Clinical records were available and reevaluated.

**Sequencing and bioinformatics analysis**

Peripheral blood was obtained from all the index patients, the relatives, and healthy controls. Genomic DNA was extracted from peripheral blood using a standard phenol–chloroform method. Primers were designed by Primer Premier 5.0 (manufactured by PREMIER Biosoft, Canada). All the coding exons of SH3TC2, PMP2, and BSCL2 were amplified by polymerase chain reaction (PCR) using 4, 16, and 8 pairs of primers, respectively. Primers and PCR conditions are available on request. Purified PCR products were sequenced by BigDye Terminator Kit 1.1 (Applied Biosystems, Foster City, CA, USA) and analyzed on ABI 3730xl automatic DNA genetic analyzer (Applied Biosystems, Foster City, CA, USA). We used following databases to obtain the frequencies of variants: dbSNP (http://www.ncbi.nlm.nih.gov/SNP), HapMap (ftp://ftp.ncbi.nlm.nih.gov/hapmap/), ExAC (http://exac.broadinstitute.org/), and 1000 Genomes Project databases (http://www.1000genomes.org/). Co-segregation analysis would be performed in family members if possible. Novel variants were further detected in Chinese healthy controls. The Clustal Omega software (manufactured by EMBL-EBI, UK). (http://www.ebi.ac.uk/Tools/msa/clustalo/) was used to investigate the conservation of the variants. Pathogenicity of the novel amino acid changes was predicted using SIFT (http://blocks.fhcrc.org/sift/SIFT.html), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), and MutationTaster (http://www.mutationtaster.org/) programs. Finally, the variants were classified according to the standards and guidelines of American College of Medical Genetics and Genomics (ACMG).[12]

**Results**

**Identification of the SH3TC2, PMP2, and BSCL2 variants**

We identified three novel heterozygous SH3TC2 variants such as p.L95V (c.283C>G), p.L1048P (c.3143T>C), and p.V1105M (c.3313G>A) in three probands [Figure 1]. The p.L95V was absent in the ExAC database and present in two Africans out of 10,000 individuals in the 1000 Genomes Project. The p.L1048P was present in one East Asian out of 121,228 individuals in the ExAC database and in two out of 10,000 individuals in the 1000 Genomes Project. The p.V1105M was absent in the 1000 Genomes Project and dbSNP databases and present in 26 individuals out of 121,398 individuals of diverse ethnicities in the ExAC database. Co-segregation analyses of these three variants were not carried out due to the unavailability of parents’ genomic DNA. These three variants were, respectively, absent in 100 healthy controls. The three nucleotides were conserved among species. They were predicted to be disease causing by SIFT, PolyPhen-2, and MutationTaster programs, except that the variant p.V1105M was predicted to be tolerated by MutationTaster. Although evaluation in silico and screening in healthy controls indicated that the three variants were likely pathogenic, no second allele variants of SH3TC2 were identified in these three patients. According to the standards and guidelines of ACMG, these three variants were considered to be of uncertain significance. In silico analysis was summarized in Table 1.

In addition, we identified two SH3TC2 variants such as c.512G>A and c.1402G>T, four PMP2 variants such as c.186A>G, c.349-30G>C, c.*12T>C, and c.*115T>C, and one BSCL2 variant c.55G>A. Population databases revealed that these variants were known polymorphisms.

**Clinical features of patients carrying SH3TC2 variants of uncertain significance**

We identified three unrelated CMT patients carrying heterozygous SH3TC2 variants, and the clinical details
were summarized in Table 2. In family 20198, patient II-1 carrying the variant p.L95V presented frequent falls as initial symptom at the age of 2. He developed foot drop at the age of 5 and was gradually unable to attend physical activities after 14 years old. Although he received orthopedic corrective surgery at 14, he did not get better. Physical examination showed that he had thenar muscles and distal lower limbs atrophy, foot dorsiflexion weakness (scored 1/5 on Medical Research Council [MRC]), reduced vibration sense, absent tendon reflexes, pes cavus, and strephenopodia. Scoliosis and cranial nerve involvement were not observed. Electrophysiological examination indicated axonal peripheral neuropathy. His CMT Neuropathy Score (CMTNS) was 15. In family 13979, patient II-1 carrying the variant p.L1048P presented cold-induced, acute-onset limbs weakness, and distal limbs numbness in an asymmetric mode as initial symptoms at 16. Disease progressed so quickly that he became unable to ambulate independently several days later. After receiving plasma exchange therapy, he got dramatic improvement but remained some sequelae including limbs weakness and atrophy. Neurological examination at 35 showed distal limbs atrophy and weakness with left limbs more seriously affected (distal upper limbs scored 3/5 and feet dorsiflexion scored 0/5 on MRC), distal limbs sensory disturbances, absent knee and ankle reflexes, and pes cavus. Electrophysiological examination indicated axonal peripheral neuropathy. His CMTNS was 16. In family 20856, patient II-1 carrying the p.V1105M presented pes cavus since infant stage. She gradually developed thenar muscles and distal lower limbs atrophy at 30. Neurological examination at 32 showed distal limbs atrophy and weakness with left limbs more seriously affected (distal upper limbs scored 3/5 and feet dorsiflexion scored 0/5 on MRC), distal limbs sensory disturbances, absent knee and ankle reflexes, and pes cavus. Electrophysiological examination revealed a demyelinating peripheral neuropathy. Her CMTNS was 16.

![Figure 1](image-url)

**Figure 1:** The pedigrees and sequencing data of the three families carrying *SH3TC2* variants of uncertain significance. The pedigree and sequence diagram of the individual carrying the *SH3TC2* p.L95V (c.283C>G) (a), the p.L1048P (c.3143T>C) (b), the p.V1105M (c.3313G>A) (c). The probands (II-1) are denoted by an arrow.

| Variants   | SIFT               | PolyPhen-2          | MutationTaster   | Clustal Omega | Population frequency | ACMG                  |
|------------|--------------------|---------------------|------------------|---------------|----------------------|-----------------------|
| p.L95V     | Affect protein function | Probably damaging | Disease causing | Conserved     | 0.0002 in 1000 Genomes | Uncertain significance |
| p.L1048P   | Affect protein function | Probably damaging | Disease causing | Conserved     | 0.0002 in 1000 Genomes | Uncertain significance |
| p.V1105M   | Affect protein function | Possibly damaging | Polymorphism     | Conserved     | 0.0002 in ExAC       | Uncertain significance |

ACMG: American College of Medical Genetics and Genomics.


**DISCUSSION**

Three novel heterozygous SH3TC2 variants such as p.L95V (c.283C>G), p.L1048P (c.3143T>C), and p.V1105M (c.3313G>A) were identified in this study. Although evaluation in silico and screening in healthy controls indicated that the three variants were likely pathogenic, no second allele variants were identified. Two out of the three patients carrying the novel SH3TC2 variants presented axonal neuropathy phenotype, and none of them exhibited SH3TC2-associated CMT clinical features, such as cranial nerve involvement, which indicated that these variants might not be pathogenic. However, we cannot rule out the possibility that there are massive deletions, duplications, or intronic variants on the second allele. Therefore, these three variants were considered to be of uncertain significance and the pathogenicity needed further studies to validate. To date, three PMP2 pathogenic variants were identified around the world.[5‑7] The PMP2 pathogenic variant p.I43N was recently identified to cause typical AD-CMT1 phenotype in forty American CMT individuals.[5] The same PMP2 variant was reported in a Korean CMT1 family.[6] Subsequently, the PMP2 pathogenic variants such as p.I52T and p.T51P were identified in a cohort of 136 European probands.[7] Our study identified no PMP2 pathogenic variants in 315 unrelated Chinese CMT families. Further analyses should be conducted in a larger cohort of Chinese CMT patients.

To date, four BSCL2 pathogenic variants such as p.N88S, p.S90L, p.S90W, and p.R96H were identified.[9,10,16,17] No BSCL2 pathogenic variants were identified in this study. Since BSCL2 pathogenic variants were related to a broad spectrum of neurological phenotypes such as Silver syndrome/spastic paraplegia 17 (SPG17) and distal hereditary motor neuropathy type V (dHMN-V), BSCL2 pathogenic variants needed to be further detected in SPG and dHMN patients in our future study.[18,19] In conclusion, SH3TC2, PMP2, and BSCL2 pathogenic variants might be rare in Chinese CMT patients and provided information for further SH3TC2 research.

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Conflicts of interest
There are no conflicts of interest.

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