Original Article

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

Xin Zhao[1], Ming-Ming Jiang[1], Yi-Zhou Yan[1], Lei Liu[2], Yong-Zhi Xie[1], Xiao-Bo Li[1], Zheng-Mao Hu[3], Xiao-Hong Zi[1], Kun Xia[3], Bei-Sha Tang[4], Ru-Xu Zhang[1]

[1]Department of Neurology, The Third Xiangya Hospital of Central South University, Changsha, Hunan 410006, China
[2]Health Management Center, The Third Xiangya Hospital of Central South University, Changsha, Hunan 410006, China
[3]State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan 410006, China
[4]Department of Neurology, The Xiangya Hospital of Central South University, Changsha, Hunan 410006, China

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 CMT1 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).

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.222331

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

Address for correspondence: Dr. Ru-Xu Zhang, Department of Neurology, The Third Xiangya Hospital of Central South University, Changsha, Hunan 410006, China
E-Mail: zhangruxu@vip.163.com

Received: 30-08-2017 **Edited by:** Li-Min Chen
How to cite this article: Zhao X, Jiang MM, Yan YZ, Liu L, Xie YZ, Li XB, Hu ZM, Zi XH, Xia K, Tang BS, Zhang RX. Screening for *SH3TC2*, *PMP2*, and *BSCL2* Variants in a Cohort of Chinese Patients with Charcot-Marie-Tooth. Chin Med J 2018;131:151-5.

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.[5-7] The clinical features of *PMP2*-associated neuropathy were similar to *PMP22* 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, 24 were AR-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

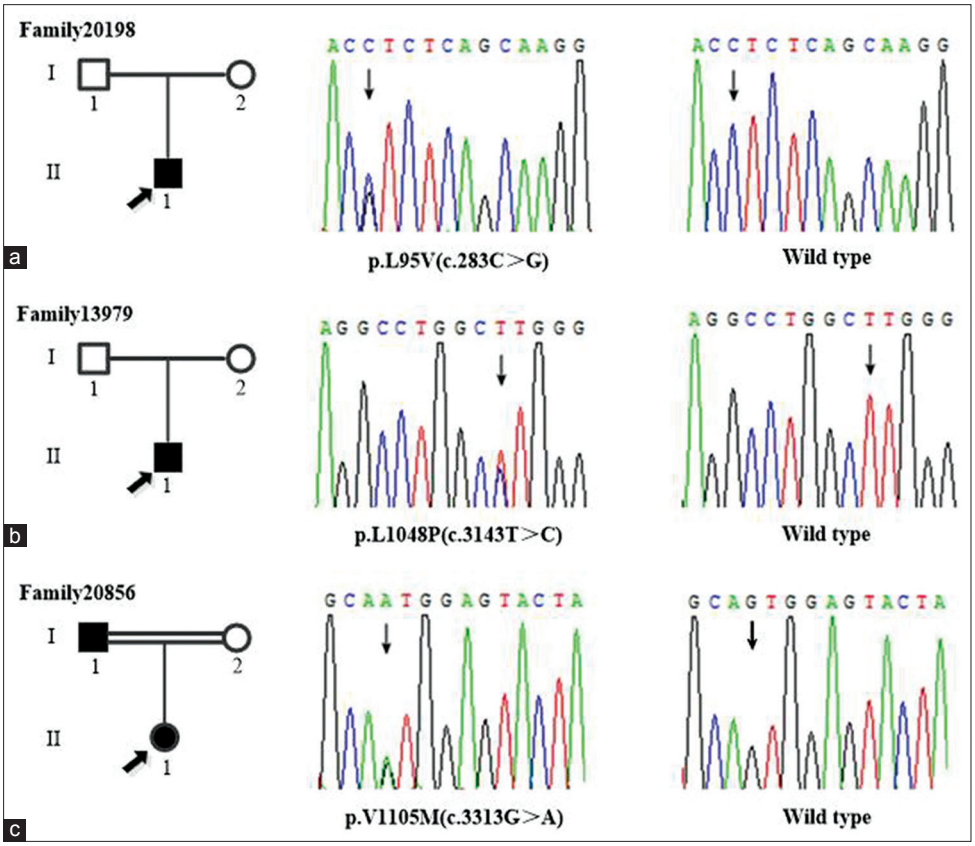

Figure 1: The pedigrees and sequencing data of the three families carrying *SH3TC2* variants of uncertain significance. The pedigree and sequence diagram of the individual carrying the *SH3TC2* p.L95V (c.283C>G) (a), the p.L1048P (c.3143T>C) (b), the p.V1105M (c.3313G>A) (c). The probands (II-1) are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

Table 1: Summary of bioinformatics analyses of the three *SH3TC2* variants of uncertain significance

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.

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, right fingers and foot dorsiflexion weakness (scored 2/5 on MRC), reduced vibration sense of both feet, absent knee and ankle reflexes, and pes cavus. Electrophysiological examination revealed a demyelinating peripheral neuropathy. Her CMTNS was 16.

Table 2: Clinical features of patients carrying the three *SH3TC2* variants of uncertain significance

Items	Family 20198	Family 13979	Family 20856
Patients	II-1	II-1	II-1
Variants	p.L95V (c.283C>G)	p.L1048P (c.3143T>C)	p.V1105M (c.3313G>A)
Sex	Male	Male	Female
Age at examination, years	18	35	32
Age at onset, years	2	16	1
Initial symptoms	Falls	Limbs weakness, distal limbs numbness	Pes cavus
Muscle weakness (UL/LL)	+ +/+ +	+ +/+ +	+ +/+ +
Sensory disturbance	V	P, T, V	V
Tendon reflex	Absent	Absent	Absent
Scoliosis	No	No	No
Cranial nerve involvement	No	No	No
Foot deformity	Pes cavus	Pes cavus	Pes cavus
Orthopedic surgery	Yes	No	No
MNCV/CMAP (m/s, mV)			
Left median	50.0/2.2	60.6/16	26.6/1.3
Right peroneus	39.4/1.3	46.2/0.3	31.8/0.4
Left peroneus	38.5/1.0	38.3/1.7	ND
SNCV/SNAP (m/s, μV)			
Left median	45.3/28.0	47.3/7.0	NR
Left peroneus superficial	NR	ND	ND
Left sural	NR	ND	NR
Right sural	NR	ND	NR
CMTNS	15	16	16

UL: Upper limb; LL: Lower limb; Muscle weakness: -: Normal; +: Distal weakness ≥4/5 on MRC; + +: Distal weakness <4/5 on MRC scale. P: Pinprick; T: Touch; V: Vibratory; MNCV: Motor nerve conduction velocity (normal range: median nerve >50 m/s, peroneal nerve >40 m/s); CMAP: Compound muscle action potential (normal range: median nerve >4 mV; peroneal nerve >2.5 mV); SNCV: Sensory nerve conduction velocity (normal range: Median nerve >45 m/s; peroneus superficial >45.5 m/s; sural nerve >36 m/s); SNAP: Sensory nerve action potential (normal range: median nerve >20 μV; peroneus superficial >10.3 μV; sural nerve >10 μV); CMT: Charcot-Marie-Tooth; CMTNS: CMT neuropathy score; NR: Not recordable; ND: Not done; MRC: Medical research council.

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, approximately forty homozygous or compound heterozygous *SH3TC2* pathogenic variants have been identified to cause AR-CMT1.[13-15] *SH3TC2*-associated CMT is mainly distributed in Mediterranean countries and accounts for about 30–57% of Mediterranean AR-CMT1 patients.[4] Only one Chinese AR-CMT1 family carrying the homozygous *SH3TC2* pathogenic variant p.E632K has been reported.[14] Together with this case report, our study added the evidence that *SH3TC2* pathogenic variants might be rare in Chinese CMT patients and provided information for further *SH3TC2* research.

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. Further studies to confirm our findings are needed.

Acknowledgments

The authors would like to thank the families for their participation in this study. The authors would like to thank all colleagues for their valuable advice in experimental procedures.

Financial support and sponsorship

This study was supported by the grants from National Natural Science Foundation of China (No. 81771366), Hunan Provincial Natural Science Foundation (No. 2017jj2365), Science Foundation of Health and Family Planning Commission of Hunan Province (No. A2017001), and Hunan Provincial Innovation Foundation for Postgraduate (No. CX2016B063).

Conflicts of interest

There are no conflicts of interest.

References

1. Skre H. Genetic and clinical aspects of Charcot-Marie-Tooth's disease. Clin Genet 1974;6:98-118. doi: 10.1111/j.1399-0004.1974.tb00638.x.
2. Harding AE, Thomas PK. Genetic aspects of hereditary motor and sensory neuropathy (types I and II). J Med Genet 1980;17:329-36. doi: 10.1136/jmg.17.5.329.
3. Senderek J, Bergmann C, Stendel C, Kirfel J, Verpoorten N, De Jonghe P, *et al.* Mutations in a gene encoding a novel SH3/TPR domain protein cause autosomal recessive Charcot-Marie-Tooth type 4C neuropathy. Am J Hum Genet 2003;73:1106-19. doi: 10.1086/379525.
4. Laššuthová P, Mazanec R, Vondráček P, Sišková D, Haberlová J, Sabová J, *et al.* High frequency of SH3TC2 mutations in Czech HMSN I patients. Clin Genet 2011;80:334-45. doi: 10.1111/j.1399-0004.2011.01640.x.
5. Gonzaga-Jauregui C, Harel T, Gambin T, Kousi M, Griffin LB, Francescatto L, *et al.* Exome sequence analysis suggests that genetic burden contributes to phenotypic variability and complex neuropathy. Cell Rep 2015;12:1169-83. doi: 10.1016/j.celrep.2015.07.023.
6. Hong YB, Joo J, Hyun YS, Kwak G, Choi YR, Yeo HK, *et al.* A mutation in PMP2 causes dominant demyelinating Charcot-Marie-Tooth neuropathy. PLoS Genet 2016;12:e1005829. doi: 10.1371/journal.pgen.1005829.
7. Motley WW, Palaima P, Yum SW, Gonzalez MA, Tao F, Wanschitz JV, *et al. De novo* PMP2 mutations in families with type 1 Charcot-Marie-Tooth disease. Brain 2016;139:1649-56. doi: 10.1093/brain/aww055.
8. Li LX, Dong HL, Xiao BG, Wu ZY. A novel missense mutation in peripheral myelin protein-22 causes Charcot-Marie-Tooth disease. Chin Med J 2017;130:1779-84. doi: 10.4103/0366-6999.211539.
9. Magré J, Delépine M, Khallouf E, Gedde-Dahl T Jr., Van Maldergem L, Sobel E, *et al*. Identification of the gene altered in berardinelli-seip congenital lipodystrophy on chromosome 11q13. Nat Genet 2001;28:365-70. doi: 10.1038/ng585.
10. Choi BO, Park MH, Chung KW, Woo HM, Koo H, Chung HK, *et al.* Clinical and histopathological study of Charcot-Marie-Tooth neuropathy with a novel S90W mutation in BSCL2. Neurogenetics 2013;14:35-42. doi: 10.1007/s10048-012-0346-5.
11. Jonghe PD, Timmerman V, Broeckhoven CV. 2nd Workshop of the European CMT Consortium: 53rd ENMC International Workshop on Classification and Diagnostic Guidelines for Charcot-Marie-Tooth Type 2 (CMT2-HMSN II) and Distal Hereditary Motor Neuropathy (distal HMN Spinal CMT) 26-28 September 1997, Naarden, the Netherlands. Neuromuscul Disord 1998;8:426-31. doi: 10.1016/S0960-8966(98)00025-X.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular pathology. Genet Med 2015;17:405-24. doi: 10.1038/gim.2015.30.
13. Piscosquito G, Saveri P, Magri S, Ciano C, Gandioli C, Morbin M, *et al.* Screening for SH3TC2 gene mutations in a series of demyelinating recessive Charcot-Marie-Tooth disease (CMT4). J Peripher Nerv Syst 2016;21:142-9. doi: 10.1111/jns.12175.
14. Yu Z, Zhang J, Xu Y, Yang B, He Z, Zhang M, *et al.* Mutation analysis for a family affected with Charcot-Marie-Tooth disease type 4C (in Chinese). Chin J Med Genet 2016;33:57-60. doi: 10.3760/cma.j.issn.1003-9406.2016.01.014.
15. Varley TL, Bourque PR, Baker SK. Phenotypic variability of CMT4C in a French-Canadian kindred. Muscle Nerve 2015;52:444-9. doi: 10.1002/mus.24640.
16. Hsiao CT, Tsai PC, Lin CC, Liu YT, Huang YH, Liao YC, *et al.* Clinical and molecular characterization of BSCL2 mutations in a Taiwanese cohort with hereditary neuropathy. PLoS One 2016;11:e0147677. doi: 10.1371/journal.pone.0147677.
17. Murphy SM, Laura M, Fawcett K, Pandraud A, Liu YT, Davidson GL, *et al.* Charcot-Marie-Tooth disease: Frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry 2012;83:706-10. doi: 10.1136/jnnp-2012-302451.
18. Auer-Grumbach M, Löscher WN, Wagner K, Petek E, Körner E, Offenbacher H, *et al.* Phenotypic and genotypic heterogeneity in hereditary motor neuronopathy type V: A clinical, electrophysiological and genetic study. Brain 2000;123(Pt 8):1612-23. doi: 10.1093/brain/123.8.1612.
19. Windpassinger C, Wagner K, Petek E, Fischer R, Auer-Grumbach M. Refinement of the silver syndrome locus on chromosome 11q12-q14 in four families and exclusion of eight candidate genes. Hum Genet 2003;114:99-109. doi: 10.1007/s00439-003-1021-6.