Mutation Analysis of GARS and Genotype-Phenotype Correlation in CMT2D/HMN5A in Chinese Patients

Bo Sun
Chinese PLA General Hospital
Zhengqing He
Chinese PLA General Hospital
Yanran Li
Chinese PLA General Hospital
Hongfen Wang
Chinese PLA General Hospital
Fei Yang
Chinese PLA General Hospital
Fang Cui
Chinese PLA General Hospital
Xu-Sheng Huang (Lewishhuang301@163.com)
Chinese PLA General Hospital https://orcid.org/0000-0002-2451-0254

Research article

Keywords: Charcot–Marie–Tooth Disease Type 2D, Hereditary Motor Neuropathy 5A, glycyl-tRNA synthetase gene

Posted Date: October 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-43866/v2

Abstract

Background

CMT2D is a rare subtype of axonal CMT, caused by a variant of the glycyl-tRNA synthetase (GARS) gene which is also a disease-causing gene of distal spinal muscular atrophy type V (dSMA-V) or hereditary motor neuropathy 5A (HMN5A). There were only several cases reported in China, all lacking an epidemiological study of CMT2D/ HMN5A.

Methods

206 patients of Chinese Han descent, clinically diagnosed with inherited peripheral neuropathy (IPN), were recruited in this study from December 20, 2012 to July 31, 2019. All patients underwent a detailed medical history screening, a neurological examination, a laboratory examination, several electrophysiological studies, and genetic testing.

Results

A total of 206 unrelated patients underwent genetic analysis. Four variants of GARS from four different families were found, including c.794C > T (p.S265F), c.374A > G (p.E125G), c.1000A > T (p.I334F), and c.781T > G (p.Y261D), with the first three being considered pathogenic. For the three pathogenic variant carriers, one was diagnosed with CMT2D, while the two others were diagnosed with HMN5A.

Conclusion

GARS mutation is a rare outcome of inherited peripheral neuropathy and the phenotype tends to be CMT2D or HMN5A.

Introduction

Inherited peripheral neuropathies (IPN) include a large heterogenous group of hereditary diseases with more than 100 causative genes reported to date. The main categories of IPN include hereditary motor and sensory neuropathy (HMSN), also called Charcot-Marie-Tooth disease (CMT), hereditary sensory and autonomic neuropathy (HSAN), and distal hereditary motor neuropathy (dHMN) [1].

Charcot–Marie–Tooth disease (CMT), the most common IPN with a worldwide incidence of 1 in 2500, comprises a group of clinically and genetically heterogeneous peripheral neuropathies [2, 3]. It is roughly classified into Type 1 (CMT1; demyelinating) and Type 2 (CMT2; axonal) according to median nerve motor conduction velocity. More than 80 genes mutations associated with CMT have been reported [4]. Owing to the development of molecular genetics, the classification of CMT has been refined.

The variant of glycyl-tRNA synthetase (GARS) gene causes Charcot–Marie–Tooth disease type 2D (CMT2D) and distal spinal muscular atrophy type V (dSMA-V), also called hereditary motor neuropathy

5A (HMN5A) [4]. In China, one family with CMT2D associated with variant of c.999G>T (p.E333D) and the other family with HMN5A associated with variant of c.383T>G (p.L128R) have been reported [5, 6], and no epidemiological study of CMT2D/HMN5A has been reported yet.

In this study, we reported four variants of GARS, including c.794C>T (p.S265F), c.374A>G (p.E125G), c.1000A>T (p.I334F) and c.781T>G (p.Y261D) in 206 unrelated Chinese Han patients with a clinical diagnosis of IPN.

Materials And Methods

Patients

A total of 206 Chinese Han patients clinically diagnosed with IPN were recruited from the Department of Neurological at the First Medical Center, of Chinese PLA General Hospital (Beijing, China) from December 20th, 2012 to March 2nd, 2020.

This study was approved by the Chinese PLA General Hospital Ethics Committee, in accordance with the principles stated in the Declaration of Helsinki. Informed consent was obtained from each patient enrolled in this study.

Electrophysiological examination

All patients underwent nerve conduction study (NCS) in which their skin temperature was maintained at 32°C or above during the examination. NCS were performed on the median, ulnar, tibial, peroneal, and sural nerves using the Keypoint electromyography (EMG) system (Medoc Ltd, Israel). The results were measured according to the normal reference values utilized by the EMG laboratory of Chinese PLA General Hospital (median motor nerve: amplitude ≥5.0 mV, velocity ≥50.0 m/s; median sensory nerve: amplitude ≥5.0 μV, velocity ≥50.0 m/s; ulnar motor nerve: amplitude ≥5.0 mV, velocity ≥50.0 m/s; ulnar sensory nerve: amplitude ≥5.0 μV, velocity ≥50.0 m/s; tibial motor nerve: amplitude ≥5.0 mV, velocity ≥40.0 m/s; peroneal motor nerve: amplitude ≥3.0 mV, velocity ≥45.0 m/s; and sural sensory nerve: amplitude ≥6.0μV, velocity ≥50.0 m/s). NCS were considered abnormal if any of the studied parameters was beyond the normal reference values [7, 8].

Sural nerve biopsy

Sural nerve biopsy was performed on Patient 1 with GARS variant with informed consent. A segment of nerve was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer with pH 7.4. Cross-sections of 1 mm thickness were post-fixed in 0.1 M osmic tetroxide for 2 h, dehydrated in a series of graded ethanols and propylene oxide, and embedded in epoxy resin (LX-112). Semithin sections were stained with toluidine blue or paragon. Thin sections were stained with lead citrate and uranyl acetate, and examined under an electron microscope [9].

Genetic analysis

All patients underwent genetic analysis by using next generation sequencing (NGS). IPN-associated genes, especially CMT-associated genes were examined. Briefly, genomic DNA was extracted from the leukocytes of fresh whole blood samples obtained from the patients. Target genes were captured by GenCap target region probe (MyGenostics Inc, Medford, MA, USA) and amplified by polymerase chain reaction. The eluted DNA was finally amplified for 15 cycles according to the following procedure: 98°C for 30 s (1 cycle), 98°C for 25 s, 65°C for 30 s, 72°C for 30 s (15 cycles), and 72°C for 5 min (1 cycle) [7, 8]. The amplified product was purified using SPRI beads (Beckman Coulter, Brea, CA, USA) according to manufacturer's protocol. Enriched libraries were sequenced using a HiSeq 2000 sequencer (Illumina, San Diego, CA, USA), which generated 100 bp paired reads [7, 8].

Depth reading of NGS identified PMP22 duplications/deletions, and multiplex ligation-dependent probe analysis (MLPA) was applied to confirm the results. Sanger direct sequencing was used to confirm and detect variants in the patients and their family members [7, 8].

The reference genome was UCSC hg19 (http://genome.ucsc.edu/). Read mapping was done by using SOAP (Short Oligonucleotide Analysis Package) aligner (http://soap.genomics.org.cn/soapaligner.html) and Burrows–Wheeler Aligner (http://bio-bwa.sourceforge.net/bwa.shtml) software [7, 8]. Variant detection included the identification of single-nucleotide polymorphisms and indels with GATK and SOAPsnp (http://soap.genomics.org.cn/soapsnp.html) software [7, 8]. The genomic variants database included the 1000 Genomes Project (browser.1000genomes.org/index.html) and the single nucleotide polymorphism database (dbSNP) (http://www.ncbi.nlm.nih.gov/projects/SNP/) [7, 8].

Bioinformatics analysis

Polymorphism Phenotyping 2 (PolyPhen-2) (http://genetics.bwh.harvard.edu/pph 2/), sorting intolerant from tolerant (SIFT) (http://sift.jcvi.org/), and Mutation Taster (http://www.mutationtaster.org/) were used to predict potential functional effects of GARS mutations [7, 8]. PolyPhen-2 classified the predicted effects of amino acid substitutions on the function of human proteins as "benign", "possibly damaging", "probably damaging" or "unknown." The functional impact of the mutation was predicted as "tolerated" or "damaging" by SIFT and as "polymorphism" or "disease-causing" by Mutation Taster [7, 8]. The pathogenicity was determined by using the ACMG guideline.

Results

Four patients, two males and two females, were identified with genetic analysis, with GARS variants (c.794C>T, p.S265F; c.374A>G, p.E125G; c.1000A>T, p.I334F and c.781T>G, p.Y261D) from 206 patients with a clinical diagnosis of IPN. The first three (c.794C>T, p.S265F; c.374A>G, p.E125G; c.1000A>T, p.I334F) of them were considered pathogenic with autosomal dominant (AD) inheritance. The mutation frequency was 1.46% (3/206).

Patient 1 was a 37-year-old male who carried c.794C>T variant (we have reported the variant in one previous study) [7] reported to cause HMN5A [10]. His bilateral distal lower limbs weakness and atrophy

started at the age of 13 and subsequently weakness of bilateral distal upper limbs developed. No subjective sensory abnormality endorsed. Physical examination showed muscle atrophy in bilateral thenar eminence, interosseous muscle, tibialis anterior muscle and calf muscle, with weakness of extremities more severe in distal lower limbs. Deep tendon reflexes were decreased, and bilateral Babinski signs were absent. Superficial sensation was lost in distal extremities while deep sensation was intact. NCS showed axonal motor neuropathy (Table 1). EMG showed active and chronic denervation potentials in both upper and lower limbs. Sural nerve biopsy showed uncompaction of myelin sheath, axonal degeneration and necrosis of peripheral nerve myelin sheath and axon (Figure 1). Patient 1 had family history with some of the family members having similar presentations (Table 2, Figure 2a). III 5, 27-year-old male, son of the proband, presented with distal lower limbs weakness at the age of 12, and then upper limbs weakness at the age of 14. Physical examination showed weakness of distal extremities and superficial sensory loss in gloving-sock pattern (Table 2). NCS of III 5 showed axonal motor neuropathy (Table 1) and EMG showed active and chronic denervation potentials in both upper and lower limbs. Sanger test confirmed that affected family members (II1, II3, II4, II6, III1 and III3) carried c.794C>T variant and unaffected members (III2 and III4) did not. According to the standards and guidelines of ACMG, the variant was considered to be pathogenic.

Patient 2 was a 34-year-old male who carried c.374A>G variant reported to cause both CMT2D and HMN5A [4, 7]. He presented with bilateral upper limbs weakness at the age of 12 and bilateral lower limbs weakness later on. No subjective sensory abnormality reported. Some family members had similar presentations, indicating autosomal dominant inheritance (figure 2b). His mother (II 8) and son (IV 2) shared c.374A>G variant confirmed by using Sanger test. Physical examination showed prominent weakness and muscle atrophy of bilateral hands and mild weakness of bilateral distal lower limbs, with decreased deep tendon reflexes and negative bilateral Babinski signs. Superficial and deep sensation were intact. NCS showed axonal motor neuropathy (Table 1). EMG showed active and chronic denervation potentials in upper and lower limbs. According to the standards and guidelines of ACMG, the variant was considered to be pathogenic.

Patient 3 was a 21-year-old female with c.1000A>T variant reported to cause HMN5A [13]. Weakness of bilateral distal lower limbs appeared about 1 year prior to evaluation. No subjective sensory abnormality was noticed. Her father and brother had similar presentations and her brother (II 2) carried the same variant (figure 2c). Physiological examination showed mild weakness and atrophy of bilateral distal upper and lower limbs. Deep tendon reflexes were decreased, and bilateral Babinski signs were absent. Superficial and deep sensation were intact. NCS showed axonal motor neuropathy (Table 1). EMG showed active and chronic denervation potentials in upper and lower limbs. According to the standards and guidelines of ACMG, the variant was considered to be pathogenic.

Patient 4 was a 38-year-old female who carried the unreported c.781T>G variant. She reported 2 months of numbness of distal extremities at the first admission in 2013. None of her family members had the same presentation. Sanger test was not performed on her family members because the unavailability of their genomic DNA. Physical examination revealed decreased superficial and deep sensation in bilateral

lower limbs, positive Romberg sign, and decreased deep tendon reflexes. Strength of extremities was normal, and bilateral Babinski signs were absent. NCS showed pure sensory neuropathy (Table 1). The variant was predicted to be damaging/probably damaging. It was also not present in the 1000 Genomes Project database. The variant was considered to be of uncertain significance according to the ACMG guideline. Other ancillary examinations including complete blood count, basic metabolic panel, vitamin B12, homocysteine, cerebral spinal fluid (CSF) test, lung CT, PET-CT etc, were within normal limits, except for positive anti-Hu antibody in serum and CSF. With 7 years' follow-up, symptoms persisted and no malignancy was detected. Reexamination of NCS showed an improvement of sensory nerve conduction (Table 1).

Discussion

In 2003, Antonellis et al. confirmed that GARS gene was the pathogenic gene of CMT2D/HMN5A for the first time [11]. Up to now, only 20 variants of GARS gene have been reported to be associated with CMT2D/HMN5A [5, 12, 13]. Classic phenotype of CMT2D/HMN5A is weakness and atrophy of upper extremities, especially the thenar eminence and the first dorsal interosseous muscle groups. The main distinguishing characteristic of the two disorders is sensory deficits in a gloving-stock pattern in patients with CMT2D. Sensory loss may vary between family members and may be normal in some. Patient 2 and Patient 3 in our study presented with classic phenotype of HMN5A, while the Patient 1 who presented with prominent superficial sensory loss in distal extremities was diagnosed as CMT2D. Upper limbs were the most frequent onset sites, and lower limbs onset (in Patient 1) could also be seen [9]. Compared with HMN5A, weakness of lower limbs in patients with CMT2D tended to be more prominent [9]. Most patients were of adolescent onset, while infant onset was also reported and tended to be more severe [14-16]. Severity of phenotype could be varying even in one family [14, 16].

Motor peripheral neuropathy in the first three patients were confirmed with electrophysiological studies. NCS of the Patient 1 and Patient 2 showed distinctively lower CMAP in median nerve than in ulnar nerve, and this imbalance involvement argued against a primary length-dependent distal axonopathy and was more in favor of a motor neuronopathy [9]. Sensory loss was the characteristic of CMT2D, and in other studies, sensory nerve action potential amplitude was decreased or diminished in CMT2D patients [6, 12]. In our study, normal sensory nerve conduction was revealed in the Patient 1, however, sural nerve biopsy showed uncompaction of the myelin sheath, axonal degeneration and necrosis of peripheral nerve myelin sheath and axon, which indicated the diagnosis of CMT2D. Not only large myelinated fibers, but also small- to middle-size fibers may occur in CMT2D [9, 16].

A Korean patient with c.794C>T (p.S265F) was diagnosed with HMN5A [17], however our patient with c.794C>T (p.S265F) was diagnosed with CMT2D, because sensory nerve damage was revealed in sural nerve biopsy [10]. Previous studies showed that the mutation c.374A>C (p.E125G) was associated with both CMT2D and HMN5A [9, 11]. Coexistence of CMT2D and HMN5A phenotypes caused by same variant remained unknown etiology. Recently, the GARS c.794C>A (p.S265Y) variant was reported in a Malian family with CMT2D [12], and c.373G>A (p.E125K) was associated to an infant patient with failure

to thrive and severe muscle weakness [18]. These two amino acid sites might be critical to GARS. Previously, c.1000A>T (p.I334F) variant was reported in a patient with HMN5A [16], and in our study, we found Patient 3, a 21-year-old female also carried c.1000A>T variant. Unlike c.794C>T (p.S265F) and c.374A>G (p.E125G) which occupied in the core catalytic domain of GARS, c.1000A>T (p.I334F) was located in the anticodon binding domain.

An unreported GARS gene variant c.781T>G (p.Y261D) was found in Patient 4. This variant was predicted as pathogenic by using different prediction tools and located in the core catalytic domain of GARS, the same as c.794C>T (p.S265F) and c.374A>G (p.E125G). However, no genetic cosegregation was found in this family and no pure sensory neuropathy associated with GARS gene variant was reported yet. According to the guidelines of ACMG, the pathogenicity of this mutation was considered indeterminate. With subacute onset of sensory neuropathy, long-term positive anti-Hu antibody, and no any other possible reason of sensory neuropathy, the patient was considered anti-Hu antibody neuropathy [19-22]. However, malignancy was not confirmed in seven-year of follow-up. Asymptomatic carriers with pathogenic variant were found in different studies [12, 23], so we cannot exclude the possibility that descendants of Patient 4 may show symptoms later. Continuous follow-up should be conducted on Patient 4 and her family members.

An American study that screened 100 patients diagnosed with dSMA, HMN, or motor axonal CMT for variants in GARS found 3 variants [16], while a Taiwan study reported two heterozygous variants found from 340 CMT patients (0.6%) [24]. Another American study showed very low frequency of GARS gene variant, only 0.4% of 3216 CMT patients [25]. In Asia, one Japanese study reported that one disease-causing mutation in one patient was found from 89 patients with axonal CMT (1.1%). [26] In this study, we found three pathogenic variants of GARS (1.46%, 3/206), including c.794C>T (p.S265F), c.374A>G (p.E125G) and c.1000A>T (p.I334F) in 206 unrelated Chinese Han patients with a clinical diagnosis of IPN (Table 3). So far, as GARS variants are rare, few studies focus on the mutation frequency of GARS. The issue of the above studies on the mutation frequency of GARS is that the inclusion criteria of patients were inconsistent, which makes horizontal comparison difficult among people from different countries and regions. Studies with larger sample size, especially multicenter studies with unified inclusion criteria of patients are required to assess the mutation frequency of GARS among different groups of people.

In general, GARS variant is a rare cause of CMT and the phenotype tends to be CMT2D or HMN5A. Clinical characteristics, NCS, and even sural nerve biopsy and skin biopsy are essential to distinguish them. As the advance of next-generation sequencing technologies including disease-specific gene panels, whole-exome sequencing and whole-genome sequencing etc, novel likely pathogenic genes and variants would be found increasingly [7]. In this study, the results suggest the importance of comprehensive assessment by using clinical phenotype, ancillary tests and genetic evidence to evaluate the pathogenicity of genetic variants in patients suspected as IPN.

Declarations

Acknowledgements

We would like to thank our colleagues for their contributions to this research work.

Funding

The study is financially supported by National Natural Science Foundation of China: The study of long non-coding RNA in the pathogenesis of CMT1A (81870989) and the study of mitochondrial unfolded protein response in the pathogenesis of CMT1B (81901274).

Conflicts of interest

There are no conflicts of interest.

References

1. Bacquet J, Stojkovic T, Boyer A, Martini N, Audic F, Chabrol B, et al. Molecular diagnosis of inherited peripheral neuropathies by targeted next-generation sequencing: molecular spectrum delineation. BMJ open. 2018;8(10):e021632.
2. Szigeti K, Lupski JR. Charcot-Marie-Tooth disease. Eur J Hum Genet. 2009;17(6):703-10.
3. Pareyson D. Charcot-marie-tooth disease and related neuropathies: molecular basis for distinction and diagnosis. Muscle Nerve. 1999;22(11):1498-509.
4. Tazir M, Hamadouche T, Nouioua S, Mathis S, Vallat J-M. Hereditary motor and sensory neuropathies or Charcot-Marie-Tooth diseases: an update. J Neurol Sci. 2014;347(1-2):14-22.
5. Yu X, Chen B, Tang H, Li W, Fu Y, Zhang Z, et al. A Novel Mutation of GARS in a Chinese Family With Distal Hereditary Motor Neuropathy Type V. Front Neurol. 2018;9:571.
6. Sun A, Liu X, Zheng M, Sun Q, Huang Y, Fan D. A novel mutation of the glycyl-tRNA synthetase (GARS) gene associated with Charcot-Marie-Tooth type 2D in a Chinese family. Neurol Res. 2015;37(9):782-7.
7. Sun B, Chen Z, Ling L, Yang F, Huang X. Clinical and genetic spectra of Charcot-Marie-Tooth disease in Chinese Han patients. J Peripher Nerv Syst. 2017;22(1):13-8.
8. Sun B, Chen ZH, Ling L, Li YF, Liu LZ, Yang F, et al. Mutation Analysis of Gap Junction Protein Beta 1 and Genotype-Phenotype Correlation in X-linked Charcot-Marie-Tooth Disease in Chinese Patients. Chinese medical journal. 2016;129(9):1011-6.
9. Sivakumar K, Kyriakides T, Puls I, Nicholson GA, Funalot B, Antonellis A, et al. Phenotypic spectrum of disorders associated with glycyl-tRNA synthetase mutations. Brain. 2005;128(Pt 10):2304-14.
10. Sun B, Li YR, Chen ZH, Ling L, Ren YT, Liu WX, et al. Analysis of a Chinese Charcot-Marie-Tooth disease type 2D pedigree. Zhonghua Yi Xue Za Zhi. 2017;97(27):2095-100.
11. Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin S-Q, et al. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. Am J

Hum Genet. 2003;72(5):1293-9.

12. Yalcouye A, Diallo SH, Coulibaly T, Cisse L, Diallo S, Samassekou O, et al. A novel mutation in the GARS gene in a Malian family with Charcot-Marie-Tooth disease. Mol Genet Genomic Med. 2019;7(7):e00782.
13. Nan H, Takaki R, Hata T, Ichinose Y, Tsuchiya M, Koh K, et al. Novel GARS mutation presenting as autosomal dominant intermediate Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2019;24(1):156-60.
14. Chung P, Northrup H, Azmath M, Mosquera RA, Moody S, Yadav A. Glycyl tRNA Synthetase (GARS) Gene Variant Causes Distal Hereditary Motor Neuropathy V. Case Rep Pediatr. 2018;2018:8516285-.
15. Eskuri JM, Stanley CM, Moore SA, Mathews KD. Infantile onset CMT2D/dSMA V in monozygotic twins due to a mutation in the anticodon-binding domain of GARS. J Peripher Nerv Syst. 2012;17(1):132-4.
16. James PA, Cader MZ, Muntoni F, Childs AM, Crow YJ, Talbot K. Severe childhood SMA and axonal CMT due to anticodon binding domain mutations in the GARS gene. Neurology. 2006;67(9):1710-2.
17. Lee HJ, Park J, Nakhro K, Park JM, Hur Y-M, Choi B-O, et al. Two novel mutations of GARS in Korean families with distal hereditary motor neuropathy type V. J Peripher Nerv Syst. 2012;17(4):418-21.
18. Forrester N, Rattihalli R, Horvath R, Maggi L, Manzur A, Fuller G, et al. Clinical and Genetic Features in a Series of Eight Unrelated Patients with Neuropathy Due to Glycyl-tRNA Synthetase (GARS) Variants. J Neuromuscul Dis. 2020.
19. Sillevis Smitt P, Grefkens J, de Leeuw B, van den Bent M, van Putten W, Hooijkaas H, et al. Survival and outcome in 73 anti-Hu positive patients with paraneoplastic encephalomyelitis/sensory neuronopathy. J Neurol. 2002;249(6):745-53.
20. Rosenblum MK. Paraneoplasia and autoimmunologic injury of the nervous system: the anti-Hu syndrome. Brain Pathol. 1993;3(3):199-212.
21. Oh SJ, Gurtekin Y, Dropcho EJ, King P, Claussen GC. Anti-Hu antibody neuropathy: a clinical, electrophysiological, and pathological study. Clin Neurophysiol. 2005;116(1):28-34.
22. Camdessanché J-P, Antoine J-C, Honnorat J, Vial C, Petiot P, Convers P, et al. Paraneoplastic peripheral neuropathy associated with anti-Hu antibodies. A clinical and electrophysiological study of 20 patients. Brain. 2002;125(Pt 1):166-75.
23. Dubourg O, Azzedine H, Yaou RB, Pouget J, Barois A, Meininger V, et al. The G526R glycyl-tRNA synthetase gene mutation in distal hereditary motor neuropathy type V. Neurology. 2006;66(11):1721-6.
24. Liao YC, Liu YT, Tsai PC, Chang CC, Huang YH, Soong BW, et al. Two Novel De Novo GARS Mutations Cause Early-Onset Axonal Charcot-Marie-Tooth Disease. PLoS One. 2015;10(8):e0133423.
25. DiVincenzo C, Elzinga CD, Medeiros AC, Karbassi I, Jones JR, Evans MC, et al. The allelic spectrum of Charcot-Marie-Tooth disease in over 17,000 individuals with neuropathy. Molecular genetics & genomic medicine. 2014;2(6):522-9.

26. Abe A, Hayasaka K. The GARS gene is rarely mutated in Japanese patients with Charcot-Marie-Tooth neuropathy. J Hum Genet. 2009;54(5):310-2.

Tables

Table 1 Nerve conduction study for patients and their affected family members

	Patient 1's family							Patient 2	Patient 3	Patient 4	
	II 1	II 3	II 4	II 6 (Patient 1)	III 1	III 3	III 5				
Left median nerve											
p. CMAP(mV)	-	-	0.9	0.7	3.3	6.1	ND	0.3	1.0	13.8	
d. CMAP(mV)	-	-	0.7	0.7	3.4	7.1	ND	0.3	1.4	16.0	
MCV(m/s)	-	-	39.2	30.6	43.5	51.3	ND	34.3	31.7	60.0	
Right median nerve											
p. CMAP(mV)	-	-	-	ND	6.3	5.4	10.8	ND	ND	ND	
d. CMAP(mV)	-	-	-	ND	7.1	6.0	11.5	ND	ND	ND	
MCV(m/s)	-	-	-	ND	56.3	54.1	35.7	ND	ND	ND	
Left ulnar nerve											
p. CMAP(mV)	0.6	2.2	8.5	6.8	11.3	5.4	ND	6.8	0.1	10.5	
d. CMAP(mV)	0.5	2.7	8.5	7.7	11.2	6.1	ND	7.9	1.8	11.2	
MCV(m/s)	37.1	41.5	59.1	43.5	52.0	52.0	ND	47.7	33.2	62.2	
Right ulnar nerve											
p. CMAP(mV)	1.5	2.7	6.4	ND	12.0	6.0	5.8	ND	ND	ND	
d. CMAP(mV)	1.5	3.4	6.2	ND	12.7	6.0	7.6	ND	ND	ND	
MCV(m/s)	33.3	38.0	59.1	ND	53.1	55.3	35.4	ND	ND	ND	
Left tibial nerve											
p. CMAP(mV)	0.6	0.8	4.8	0.5	1.6	0.8	1.2	0.3	1.1	9.0	
d. CMAP(mV)	0.4	1.2	4.8	0.4	1.5	0.7	0.3	0.3	1.2	10.4	
MCV(m/s)	43.8	41.8	40.0	38.1	40.9	36.0	22.7	39.6	43.3	43.8	
Right tibial nerve											
p. CMAP(mV)	0.4	1.2	3.1	0.4	1.8	2.4	0.1	0.5	0.8	ND	
d. CMAP(mV)	0.4	1.7	3.8	0.4	1.9	2.5	0.1	0.5	0.7	ND	
MCV(m/s)	44.8	38.9	40.0	40.0	45.0	42.4	39.0	42.0	40.3	ND	
Left peroneal nerve											
p. CMAP(mV)	-	-	0.1	0.1	1.6	0.1	0.5	0.2	-	3.6	
d. CMAP(mV)	-	-	0.1	0.6	1.4	0.1	0.5	0.2	-	3.7	
MCV(m/s)	-	-	35.3	28.1	39.0	29.4	29.6	31.0	-	44.4	
Right peroneal nerve											
p. CMAP(mV)	-	-	0.1	-	0.3	0.1	0.1	0.5	-	4.7	
d. CMAP(mV)	-	-	0.1	-	0.4	0.1	0.2	0.4	-	4.5	
MCV(m/s)	-	-	30.6	-	35.7	39.5	20.6	43.8	-	50.0	
Left median nerve											
SNAP(μV)	ND	12.0	15.0	9.5	16.0	15.0	ND	4.6	68.3	-	2.8*
SCV(m/s)	ND	56.0	60.0	57.1	65.2	60.0	ND	50.0	65.7	-	63.6*
Right median nerve											
SNAP(μV)	8.2	11.0	12.0	ND	13.0	23.0	5.6	ND	ND	-	-*
SCV(m/s)	55.5	54.5	68.2	ND	68.2	62.5	55.2	ND	ND	-	-*
Left ulnar nerve											
SNAP(μV)	ND	7.8	9.8	7.7	6.8	9.1	ND	6.3	51.4	14.0	3.3*
SCV(m/s)	ND	51.0	54.5	53.8	60.0	54.8	ND	52.0	58.3	54.5	57.9*
Right ulnar nerve											
SNAP(μV)	4.3	8.9	15.0	ND	10.0	7.3	3.7	ND	ND	4.0	2.4*
SCV(m/s)	56.5	52.7	54.5	ND	60.0	54.4	46.7	ND	ND	57.1	61.5*
Left sural nerve											

SNAP(μV)	6.3	11.0	17.0	10.0	7.4	9.1	12.0	9.4	8.3	-	3.0*
SCV(m/s)	55.5	55.0	55.6	55.6	65.2	52.7	50.0	50.0	43.9	-	58.3*
Right sural nerve											
SNAP(μV)	4.0	13.0	11.0	16.0	7.8	8.8	13.0	14.0	8.4	-	-*
SCV(m/s)	62.5	53.0	62.5	53.6	60.0	59.6	51.7	51.7	40.6	-	-*

Note: p.: proximal, d.: distal, CMAP: compound muscle active potential, SNAP: sensory nerve active potential, MCV: motor conduction velocity, SCV: sensory conduction velocity, ND: not done, —: no respond, *: the second test of Patient 4.

Table 2 Clinical characteristics for Patient 1's family members

	II 1	II 3	II 4	II 6 (Patient 1)	III 1	III 3	III 5
Gender	M	M	F	M	F	F	M
Onset age (years)	14	13	12	13	14	13	12
Examination age (years)	45	43	39	37	18	16	14
Onset site	LL	LL	UL	LL	UL&LL	LL	LL
Subjective motor deficit	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL
Strength deficit on examination	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL
Muscle atropy	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL
Subjective sensory abnormality	+	+	—	+	+	+	+
Superficial sensation deficit	+	+	—	+	+	+	+
Deep sensation deficit	—	—	—	—	+	—	—
Areflexes	+	+	+	+	+	+	+
Babinski sign	—	—	—	—	—	—	—

Note: UL: upper limbs; LL: Lower limbs;+: present; —:absent;

Table 3 Mutation frequency of GARS in patients from different countries and regions

Year	Reference	Country/Area	Inclusive patient	Frequency	Variant/Phenotype	de novo variant
2020	This study	Chinese mainland	CMT	1.5% (3/206)	p.S265F/CMT2D; p.E125G/dSMA-V; p.I334F/dSMA-V	-
2015	Yi-Chu Liao et al	Taiwan	CMT	0.6% (2/340)	p.M292R/CMT2D; p.D200Y/CMT2D	2
2014	Christina DiVincenzo et al	America	CMT	0.4%	UN	UN
2009	Akiko Abe et al	Japan	axonal CMT	1.1% (1/89)	p.P298L/CMT2D	-
2006	P.A. James et al	UK	dSMA, HMN, or motor axonal CMT	3% (3/100)	p.G652A/iSMA; p.S635L/CMT2D; p.I334F/dSMA-V	1

Note: CMT:Charcot–Marie–Tooth disease, dSMA:distal spinal muscular atrophy; iSMA:infant-onset spinal muscular atrophy; HMN:hereditary motor neuropathy; -:absent; UN:unknown

Figures

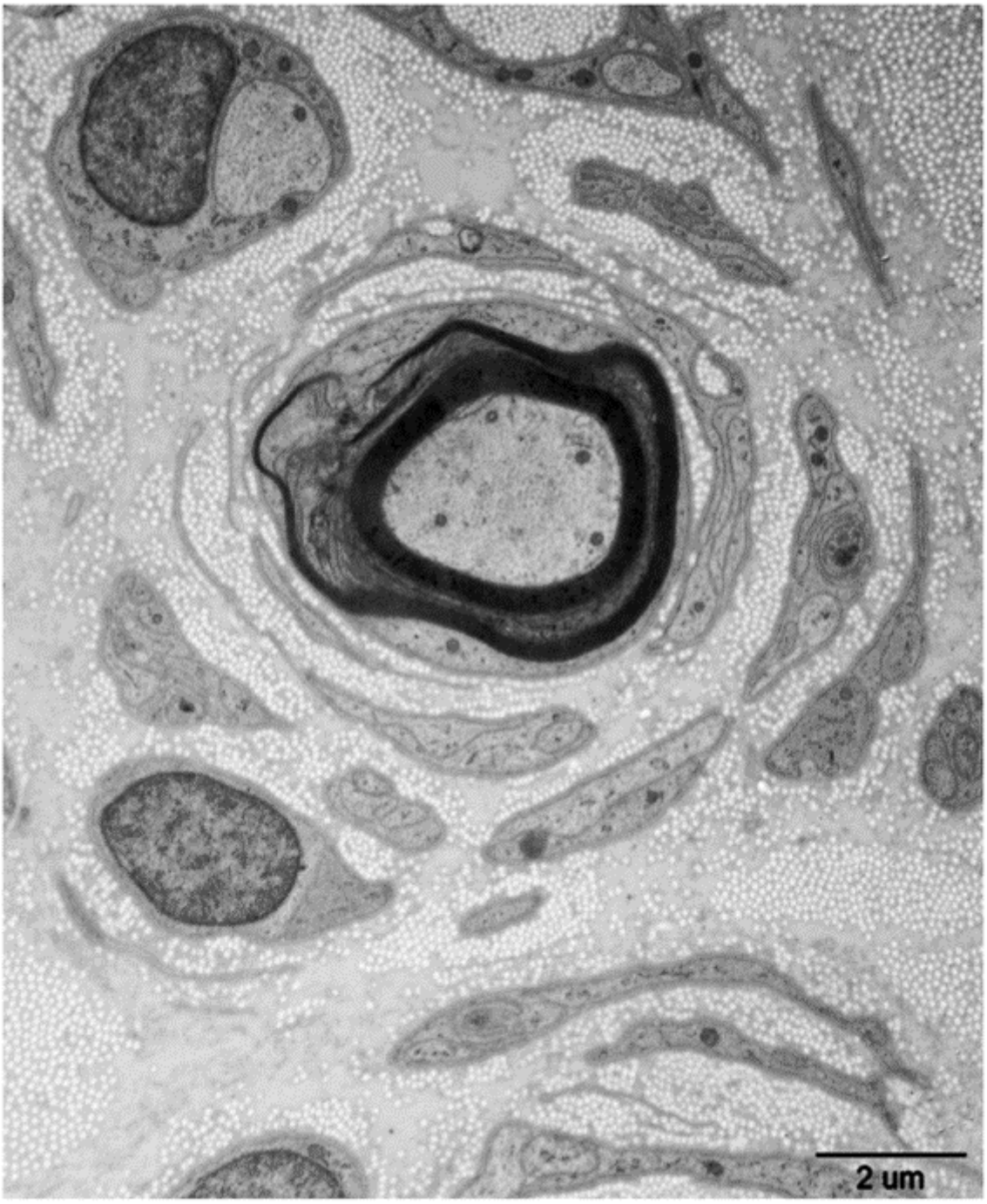
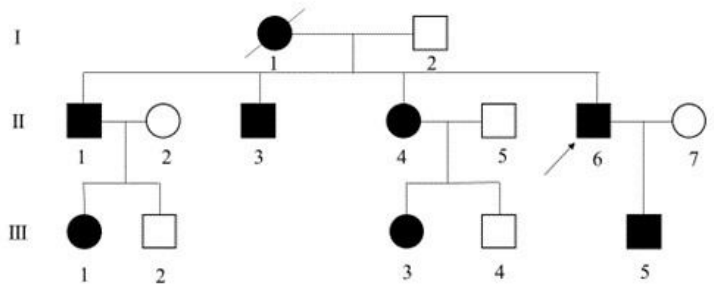
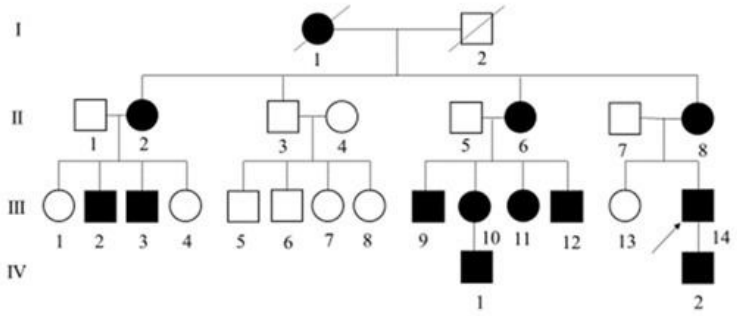


Figure 1

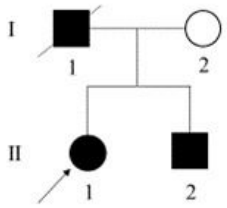
right sural biopsy of the patient with c.794C>T, p.S265F mutation. Sural nerve biopsy showed uncompaction of myelin sheath, axonal degeneration and necrosis of peripheral nerve myelin sheath and axon.



2a



2b



2c

Figure 2

a, b and c. Pedigrees of the three families with the c.794C>T(1a), c.374A>G(1b) and c.1000A>T(1c) GARS gene mutation. Square = male; circle = female; diagonal black line = deceased individual; black filled symbol = affected individual; empty symbol = clinically healthy relative