Screening for GARS Variants in A Cohort of Chinese Patients With Inherited Peripheral Neuropathy

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Abstract

Background CMT2D is a rare subtype of axonal CMT, caused by the mutation of 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 case reports in China, and no epidemiological study of CMT2D/ HMN5A yet.

Methods We recruited the patients of Chinese Han descent clinically diagnosed with inherited peripheral neuropathy (IPN) from the Department of Neurology at Chinese PLA General Hospital (Beijing, China) from December 20, 2012 to July 31, 2019. All patients underwent a detailed medical history, neurological examination, laboratory examination, electrophysiological studies, and genetic testing.

Results A total of 206 unrelated patients underwent genetic analysis, and we found four mutations of GARS from four different families, including c.794C>T (p.S265F), c.374A>G (p.E125G), c.1000A>T (p.I334F) and c.781T>G (p.Y261D), the first three of them were considered pathogenic. As for the three pathogenic mutation carriers, one patient was diagnosed as CMT2D, two patients were diagnosed as HMN5A.

Conclusion GARS mutation is a rare cause of inherited peripheral neuropathy and the phenotype tends to be CMT2D or HMN5A. There might be a relatively higher mutation frequency in Asian population compared with Caucasians. Combination of clinical phenotype, auxiliary tests and genetic evidence to assess the pathogenicity of genetic variants in patients suspected as IPN is of vital importance.

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], and is roughly classified into Type 1 (CMT1; demyelinating) and Type 2 (CMT2; axonal) according to median nerve motor conduction velocity. More than 80 genes have been reported as being associated with CMT [4]. Owing to the development of molecular genetics, the classification of CMT is refined.

The mutation 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 Chinese Mainland, one family with CMT2D caused by mutation of c.999G > T (p.E333D) and the other family with HMN5A caused by mutation of c.383T > G (p.L128R) have been reported [5, 6], and no epidemiological study of CMT2D/HMN5A was 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 unrelated Chinese Han patients clinically diagnosed with IPN were recruited from the Neurological Department of the First Medical Center, Chinese PLA General Hospital (Beijing, China) from December 20th, 2012 to March 2nd, 2020. Patients underwent detailed history-taking, neurological examination, laboratory examination, electrophysiological studies, and genetic testing.

This study was approved by the Chinese PLA General Hospital Ethics Committee, in accordance with the principles stated in the Declaration of Helsinki. Informed written 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 found to be abnormal [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 buffered to pH 7.4 with 0.1 M phosphate buffer. 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 via NGS (high throughput target sequencing). We examined IPN-associated genes, especially CMT-associated genes (Table 1). Genomic DNA was extracted from the peripheral leukocytes of fresh blood samples obtained from patients with a clinical diagnosis of IPN. 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 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 using 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 the ACMG guideline.

Table 1
List of examined Charcot–Marie–Tooth disease-associated genes

PMP22	SBF2	EGR2	HSPB8	HSPB1	DYNC1H1	DNAJB2
MPZ	SBF1	PRX	HSPB1	GDAP1	MYH14	INF2
LITAF	RAB7A	HK1	HSPB3	CCT5	TRPV4	GNB4
EGR2	DHTKD1	FGD4	SETX	PRNP	AARS	YARS
NEFL	TRIM2	Figure 4	DNAJB2	NGF	SPTLC1	KARS
FBLN5	PDK3	CTDP1	BSCL2	HSPB8	SPTLC2	PLEKHG5
GDAP1	AIFM1	KIF1B	GARS	DNM2	RAB7	GJB1
MTMR2	MARS	MFN2	REEP1	AARS	ATL1	PRPS1
SH3TC2	HARS	LMNA	IGHMBP2	LRSAM1	DNMT1	HOXD10
NDRG1	HINT1	MED25	SLC5A7	KIF5A	WNK1	IKBKAP
KIF1A	TFG	NTRK1	DCTN1	DNM2	FAM134B	SCN9A
NEFL	BICD2	DCTN1	ATP7A			

Results

Genetic analysis identified four patients, two males and two females, with GARS mutations (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 and the mutation frequency was 1.46% (3/206). Clinical characteristics of patients and their affected family members were summarized in Table 2.

Table 2
Clinical characteristics of 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			
Mutation type	c.794C > T	c.794C > T	c.794C > T	c.794C > T	c.794C > T	c.794C > T	c.794C > T	c.374A > G	c.1000A > T	c.781T > G
	(p.S265F)	(p.S265F)	(p.S265F)	(p.S265F)	(p.S265F)	(p.S265F)	(p.S265F)	(p.E125G)	(p.I334F)	(p.Y261D)
Gender	M	M	F	M	F	F	M	M	F	F
Onset age (years)	14	13	12	13	14	13	12	12	20	38
Examination age (years)	45	43	39	50	18	16	14	34	21	38
Onset site	LL	LL	UL	LL	UL&LL	LL	LL	UL	UL	UL&LL
Subjective motor deficit	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL&LL	UL	—
Strength deficit on examination	UL&LL	UL&LL	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	UL&LL	UL	—
Subjective sensory abnormality	—	—	—	—	—	—	—	—	—	+
Superficial sensation deficit on examination	+	+	—	+	+	+	+	—	—	+
Deep sensation deficit on examination	—	—	—	—	+	—	—	—	—	+
Areflexes	+	+	+	+	+	+	+	+	+	+
Babinski sign	—	—	—	—	—	—	—	—	—	—
Note: UL: upper limbs; LL: Lower limbs; +: present; —: absent;										

Patient 1 was a 37-year-old male who carried c.794C > T variant reported to cause HMN5A [10]. He presented with bilateral distal lower limbs weakness and atrophy at the age of 13 and subsequently presented with weakness of bilateral distal upper limbs. No subjective sensory abnormality was found. Physiological examination showed muscle atrophy in bilateral thenar eminence, interosseous muscle, tibialis anterior muscle and calf muscle. Weakness of extremities, more severe in distal lower limbs, were found. Deep tendon reflexes were decreased, and bilateral Babinski signs were negative. Superficial sensation was lost in distal extremities with intact deep sensation (Table 2). NCS showed axonal motor neuropathy (Table 3). EMG showed active and chronic denervation potentials in both upper and lower limbs. Sural nerve biopsy showed unclear laminar structure in myelin sheath, axonal degeneration and necrosis of peripheral nerve myelin sheath and axon (Fig. 2). Patient 1 had positive family history and some of the family members had similar presentation (Table 2, Fig. 1a). III 5, 27-year-old male, son of the proband, presented with distal lower limbs weakness at the age of 12, then upper limbs weakness at the age of 14. Physiological examination showed weakness of distal extremities and superficial sensory loss in gloving and socking pattern (Table 2). NCS of III 5 showed axonal motor neuropathy (Table 3) 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 mutation and unaffected members (III2 and III4) did not. The variant was predicted to be pathogenic by SIFT, PolyPhen-2, and Mutation Taster. It was also not present in the 1000 Genomes Project database. According to the standards and guidelines of ACMG, the variant was considered to be pathogenic.

Table 3
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											

	Patient 1's family							Patient 2	Patient 3	Patient 4	
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.

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 presented with bilateral lower limbs weakness later. No subjective sensory abnormality was reported. Some family members had similar presentation,indicating autosomal dominant inheritance (Fig. 1b). Sanger test was performed on his mother (II 8) and son (IV 2), and the c.374A > G mutation was found. Physiological examination showed prominent weakness and muscle atrophy of bilateral hands. Mild weakness of bilateral distal lower limbs was also found. Deep tendon reflexes were decreased, and bilateral Babinski signs were negative (Table 2). Superficial and deep sensation were intact. NCS showed axonal motor neuropathy (Table 3). EMG showed active and chronic denervation potentials in upper and lower limbs. The variant was predicted to be pathogenic by SIFT, PolyPhen-2, and Mutation Taster. It was also not present in the 1000 Genomes Project database. According to the standards and guidelines of ACMG, the variant was considered to be pathogenic.

Patient 3 was a 21-year-old female who carried c.1000A > T variant reported to cause HMN5A [13]. Weakness of bilateral distal lower limbs appeared about 1 year ago. No subjective sensory abnormality was found. Her father and brother had similar presentation and her brother (II 2) carried the same mutation (Fig. 1c). Physiological examination showed mild weakness and muscle atrophy of bilateral distal upper and lower limbs. Deep tendon reflexes were decreased, and bilateral Babinski signs were negative (Table 2). Superficial and deep sensation were intact. NCS showed axonal motor neuropathy (Table 3). EMG showed active and chronic denervation potentials in upper and lower limbs. The variant was predicted to be pathogenic by SIFT, PolyPhen-2, and Mutation Taster. It was also not present in the 1000 Genomes Project database. 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 c.781T > G variant which was not reported before. She presented with numbness of distal extremities for 2 months at the first admission to our hospital in 2013. There were no other patients in her family and no genetic cosegregation presented in this family. Physiological examination revealed decreased superficial and deep sensation in bilateral lower limbs, positive Romberg sign, and decreased deep tendon reflexes. Strength of extremities were normal, and bilateral Babinski signs were negative (Table 2). NCS showed sensory neuropathy with intact motor nerve conduction (Table 3). EMG of upper and lower limbs was normal. The variant was predicted to be damaging/probably damaging by SIFT, PolyPhen-2, and Mutation Taster. It was also not present in the 1000 Genomes Project database. According to the standards and guidelines of ACMG, the variant was considered to be of uncertain significance. Other auxiliary examinations including blood routine test, blood glucose, liver function, renal function, vitamin B12, homocysteine, routine cerebral spinal fluid (CSF) test, lung CT and PET-CT etc, were normal, except for positive anti-Hu antibody in serum and CSF. With 7 years' follow-up, no malignancy was found and symptoms persisted. Reexamination of NCS showed an improvement of sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) in left median and bilateral sural nerves and decreased SNAP amplitude in bilateral ulnar nerves (Table 3).

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 mutations 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 stocking and gloving pattern in patients with CMT2D. While sensory loss may vary from family members: sensation may be normal in some family members. 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 of 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 adolescent onset, while infant onset was also reported and tended to be more severe [14–16]. Severity of phenotype could be various even in one family [14, 16].

The results of electrophysiological studies confirmed motor peripheral neuropathy in the first three patients. 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]. However, we did not think normal sensory nerve conduction study could deny the diagnosis of CMT2D for Patient 1, for sensory nerve degeneration in CMT2D could involve small-size and middle-size fibers, sparing large myelinated fibers [9, 16], and sural nerve biopsy showed unclear laminar structure in myelin sheath, axonal degeneration and necrosis of peripheral nerve myelin sheath and axon.

A Korean patient with c.794C > T (p.S265F) was diagnosed as HMN5A [17], however our patient with c.794C > T (p.S265F) was diagnosed as 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 mutation remained unknown etiology. Recently, the GARS c.794C > A (p.S265Y) mutation 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) mutation 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 mutation c.781T > G (p.Y261D) was found in Patient 4. This mutation was predicted as pathogenic by different prediction tools and was 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 presented in this family and no pure sensory neuropathy associated with GARS gene mutation was reported yet. According to the guidelines of ACMG, the pathogenicity of this mutation was not considered. Due to subacute onset sensory neuropathy, long-term positive anti-Hu antibody, and no any other possible reason of sensory neuropathy, the patient was diagnosed as anti-Hu antibody neuropathy. Anti-Hu antibody is a paraneoplastic marker associated with peripheral and central nervous system disturbances, such as subacute sensory neuropathy, cerebellar dysfunction, limbic encephalitis and motor neuron disease etc [19, 20]. Considering anti-Hu antibody combined with nervous system lesion had a strong connection with tumor especially small cell lung cancer [19, 21, 22], and in some cases neurological symptoms presented ahead of tumor diagnosis [19], consecutive follow-up for Patient 4 was necessary. Asymptomatic carriers with pathogenic variation were found in different studies [12, 23], so we cannot exclude the possibility that descendants of Patient 4 may show symptoms later. But based on current evidence, we should not tell the Patient 4's family this possibility that may increase unnecessary psychological burden.

An American study that screened 100 patients diagnosed with dSMA, HMN, or motor axonal CMT for mutations in GARS found 3 mutations [16], while a Taiwan study reported two heterozygous mutations found from 54 axonal CMT patients indicating a higher mutation frequency [24]. Another American study showed very low frequency of GARS gene mutation, only 0.4% of 3216 CMT patients [25]. In this study, we found three pathogenic mutations 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.

In general, GARS mutation 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 mutations would be found increasingly. In this study, we suggest the importance on combining clinical phenotype, auxiliary tests and genetic evidence to assess the pathogenicity of genetic variants in patients suspected as IPN.

Declarations

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Conflicts of interest

There are no conflicts of interest.

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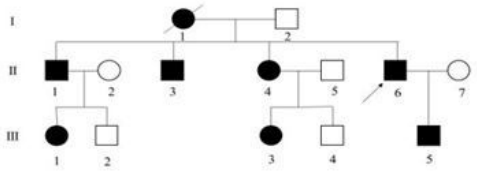
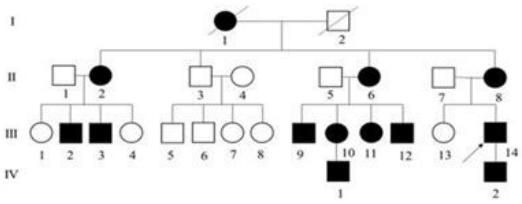
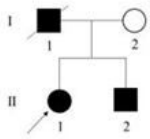
Figures



1a



1b



1c

Figure 1

1a, 1b and 1c. 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

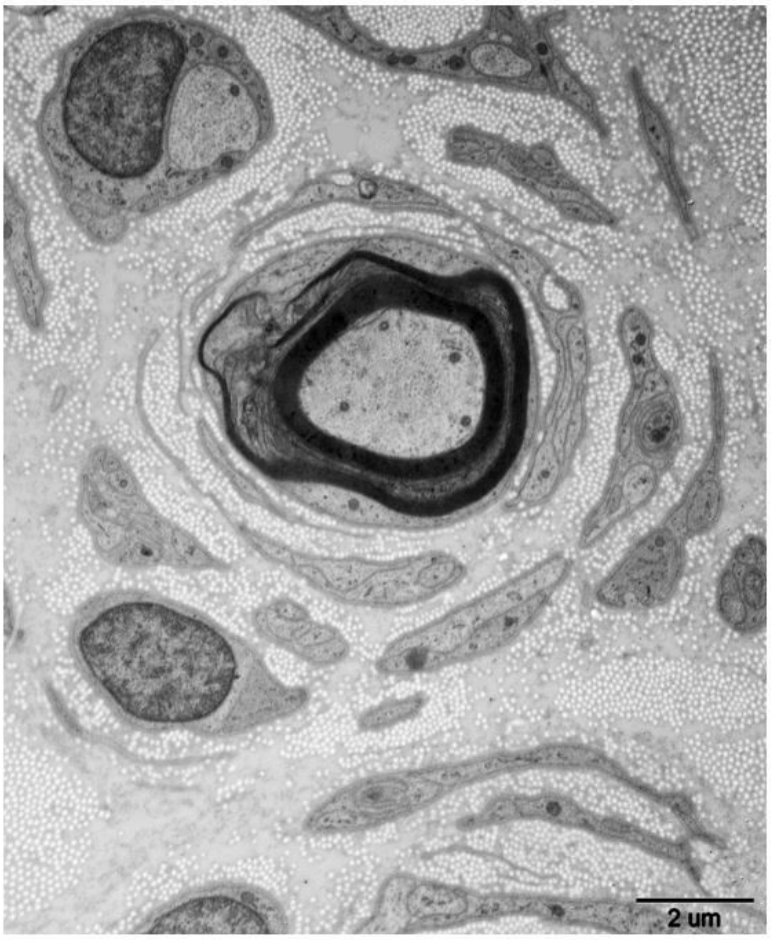


Figure 2

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