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Episodic Neurological Dysfunction in X-Linked Charcot-Marie-Tooth Disease: Expansion of the Phenotypic and Genetic Spectrum

Feixia Zhan[a]
Wotu Tian[a]
Yuwen Cao[a]
Jingying Wu[a]
Ruilong Ni[a,b]
Taotao Liu[a,b]
Yun Yuan[c]
Xinghua Luan[a]
Li Cao[a,b]

[a]Department of Neurology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b]School of Medicine, Anhui University of Science and Technology, Huainan, China
[c]Department of Neurology, Peking University First Hospital, Beijing, China

Background and Purpose X-linked Charcot-Marie-Tooth disease type 1 (CMTX1) is characterized by peripheral neuropathy with or without episodic neurological dysfunction. We performed clinical, neuropathological, and genetic investigations of a series of patients with mutations of the gap-junction beta-1 gene (*GJB1*) to extend the phenotypic and genetic description of CMTX1.

Methods Detailed clinical evaluations, sural nerve biopsy, and genetic analysis were applied to patients with CMTX1.

Results We collected 27 patients with CMTX1 with *GJB1* mutations from 14 unrelated families. The age at onset (AAO) was 20.9±12.2 years (mean±standard deviation; range, 2−45 years). Walking difficulties, weakness in the legs, and pes cavus were common initial symptoms. Compared with female patients, males tended to have a younger AAO (males vs. females=15.4±9.6 vs. 32.0±8.8 years, p=0.002), a longer disease course (16.8±16.1 vs. 5.5±3.8 years, p=0.034), and more-severe electrophysiological results. Besides peripheral neuropathy, six of the patients had special episodic central nervous system (CNS) evidence from symptoms, signs, and/or reversible white-matter lesions. Neuropathology revealed the loss of large myelinated fibers, increased number of regenerated axon clusters with abnormally thin myelin sheaths, and excessively folded myelin. Genetic analysis identified 14 *GJB1* variants, 6 of which were novel.

Conclusions These findings expand the phenotypic and genetic spectrum of CMTX1. Although CMTX1 was found to have high phenotypic and CNS involvement variabilities, detailed neurological examinations and nerve conduction studies will provide critical clues for accurate diagnoses. Further exploration of the underlying mechanisms of connexin 32 involvement in neuropathy or CNS dysfunction is warranted to develop promising therapies.

Keywords X-linked Charcot-Marie-Tooth disease; CMTX1; episodic neurological dysfunction; *GJB1*.

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Correspondence
Li Cao, MD, PhD
Department of Neurology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 600 Yishan Road, Shanghai 200233, China
Tel +86-021-24056187
Fax +86-021-24056187
E-mail caoli2000@yeah.net

Xinghua Luan, MD
Department of Neurology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 600 Yishan Road, Shanghai 200233, China
Tel +86-021-24056187
Fax +86-021-24056187
E-mail green_lxh@hotmail.com

INTRODUCTION

Charcot-Marie-Tooth disease (CMT), also referred to as hereditary motor and sensory neuropathy, encompasses a group of clinically and genetically heterogeneous peripheral neuropathies and has a reported overall prevalence of 1−4/10,000.[1] CMT is classically characterized by progressive length-dependent motor and sensory defects that start at the distal extremities with skeletal deformities, and loss of deep tendon reflexes.[2] CMT can be clinically subdivided into demyelinating (CMT1), axonal (CMT2), and intermediate forms based on electrophysiological characteristics.[3] More than 100 genes are known to cause CMT, with autosomal dominant, autosomal recessive, or X-linked inheritance.[4] X-linked

CMT type 1 (CMTX1) is the second most common subtype of CMT, accounting for 10%−20% of all cases.[5,6] Connexin 32 (Cx32) is encoded by the gap-junction beta-1 (*GJB1*) and expressed by many tissues, including myelinating Schwann cells and oligodendrocytes. Patients with *GJB1* variants manifest both peripheral and central nervous system (CNS) involvement.[7,8] More than 500 *GJB1* mutations have been identified as related to CMTX1 (https://neuropathybrowser.zuchnerlab.net/#/). Cx32 participates in the formation of gap junctions between Schwann and their adjacent cells that mostly localize at the noncompact myelin of incisures and paranodes, providing a channel for the exchange of ions and small molecules across the myelin sheath.[9] These gap junctions therefore play a crucial role in the homeostasis of myelinated axons as they are involved in diverse biological functions, including metabolic cooperation, spatial buffering, electrical signaling, growth control, and cellular differentiation.[10] Although several studies have investigated the possible mechanisms of different *GJB1* mutants, the exact pathogenesis is yet to be fully understood.

This study investigated the phenotypic, neuropathological, and genotypic features of CMTX1 in a cohort of 27 Chinese patients. *GJB1*-associated CMTX1 was found to have high phenotypic and CNS involvement variabilities. Six novel *GJB1* mutations were identified in this study, thereby expanding the genetic spectrum known to be associated with CMTX1.

METHODS

Patients

We collected 27 patients with CMTX1 and *GJB1* mutations from 14 unrelated families in this study. Clinical phenotype, neuroelectrophysiology, and brain images were extensively evaluated by at least two senior neurologists. The clinical symptoms of CMTX1 were evaluated using the Charcot-Marie-Tooth disease neuropathy score version 2 (CMTNS). The study was approved by the ethics committee of Shanghai Sixth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China (IRB No. 2021-219). Each patient or their guardians provided written informed consent.

Neuropathology

A sural nerve biopsy was performed on several probands. Tissue sections were stained using the conventional histological methods of hematoxylin and eosin, luxol fast blue, and Congo red. Separate portions of the specimen were fixed in a 2% paraformaldehyde/2.5% glutaraldehyde phosphate buffer, processed, and embedded in Epon. Transverse semithin sections were stained using toluidine blue and evaluated using a light microscope. The ultrastructure was studied by contrasting ultrathin sections with uranyl acetate and lead citrate and examining them using an electron microscope.

Genetic analyses

Genomic DNA was extracted from peripheral blood using the standard protocol. Patients with PMP22 duplications or deletions were initially excluded by applying the multiplex ligation-dependent probe amplification technique to demyelinating and intermediate CMT. Exome sequencing was performed using SureSelect v6 reagents (Agilent) to capture exons and the HiSeq X Ten platform (Illumina) for sequencing. *GJB1* variants with minor allele frequencies higher than 5%, as reported in public databases (1,000 Genomes Project, dbSNP, and gnomAD), were excluded first. Variants were then screened using 200 healthy controls of Han Chinese ethnicity, and in silico analyses were performed using MutationTaster (http://www.mutationtaster.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (https://sift.bii.a-star.edu.sg/), and Combined Annotation-Dependent Depletion (CADD) scores. Sanger sequencing and cosegregation analysis were further applied to family members of the patients. The pathogeneses of the variants were interpreted and classified according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines.[11]

RESULTS

Clinical and electrophysiological data

This study detected that 27 patients with CMTX1 (15 males and 12 females) carried *GJB1* mutations. The detailed clinical data of each patient are listed in Supplementary Table 1 (in the online-only Data Supplement). The age at onset (AAO) was 20.9±12.2 years (mean±standard deviation; range, 2−45 years). More than half of the patients (54.5%, 12/22) first exhibited symptoms before the age of 20 years. The disease duration was 13.2±14.3 years (range, 1−55 years). Gait abnormalities, walking difficulties, weakness in the legs, and pes cavus were common initial clinical symptoms. Most patients presented typical phenotypes, including distal-limb weakness (77.8%), foot deformity (70.4%), and sensory disturbance (48.1%). Only two patients (F1−II:1, F6−III:2) had a fine tremor. We collected electrophysiological data from 20 patients; the other 7 did not undergo the electrophysiological evaluation for specific reasons such as fear of pain or having no symptoms. Based on a cutoff median motor nerve conduction velocity (MNCV) of 38 m/s at the time of examination, the phenotypes of *GJB1* mutations consisted of 12 patients with CMT1 and 8 with CMT2. Meanwhile, 80% of the patients (16/20) had intermediate MNCVs of 35−45 m/s. The

CMTNS score was 10.6±4.6 (range, 2–18). The detailed clinical manifestations and electrophysiological data of all patients with CMTX1 are listed in Table 1.

Comparing sex differences in phenotypes revealed that female patients had various clinical manifestations ranging from mild to relatively severe, whereas male patients tended to have a younger AAO (males vs. females=15.4±9.6 vs. 32.0±8.8 years, p=0.002) and longer disease course (males vs. females=16.8±16.1 vs. 5.5±3.8 years, p=0.034). Regarding electrophysiological data at the time of examination, the male patients had a trend to exhibit a lower median MNCV (males vs. females=36.1±6.9 vs. 39.5±7.9 m/s), lower median compound muscle action potential (CMAP) (males vs. females=2.5±2.1 vs. 3.0±1.2 mV), and higher CMTNS (males vs. females=12.4±3.5 vs. 8.4±4.8).

CNS manifestations of CMTX1

Besides peripheral neuropathy, CNS involvement was also observed in our study. Three patients presented transient paroxysmal symptoms (patients F4–II:1, F9–II:2, and F10–III:6), three had abnormal white-matter signals on MRI (F4–II:1, F10–III:6, and F13–III:1), one (F8–III:1) had only abnormal signs (horizontal nystagmus and gait ataxia), and one (F7–III:3) only presented prolonged latencies of brainstem auditory evoked potentials. The abnormal brainstem auditory evoked potentials manifested as significantly prolonged latencies of binaural III wave (left=4.35 ms, right=4.28 ms), as well as the interwave latency of I to III wave (left=2.97 ms, right=2.85 ms). Patient F4–II:1 was a 14-year-old male who had experienced several clusters of episodic lingual paralysis, dysphasia, limb weakness, and numbness during the previous 2 years, which were easily induced by a cold or fever. Each attack recovered after about 2 hours. A physical examination revealed distal-limb muscle atrophy, sensory loss, areflexia, and positive Babinski signs. Oligoclonal bands were positive in both serum and cerebrospinal fluid. Electrophysiological studies revealed mixed polyneuropathy that involved motor and sensory nerves. Brain MRI revealed abnormal signals in the splenium of the corpus callosum and posterior limbs of the bilateral internal capsule, while the lesions significantly improved after 6 months (Fig. 1A, upper line). Patient F9–II:2, a 34-year-old female, complained of progressive walking instability with a 12-year history. Except for the typical symptoms of peripheral neuropathy, she also had a history of recurrent epileptic seizures since the age of 10 years. Each typical seizure episode was characterized

Table 1. Clinical and electrophysiological data of all patients with X-linked Charcot-Marie-Tooth disease type 1 and gap-junction beta-1 mutations

Clinical symptoms and signs	Total (n=27)	Males (n=15)	Females (n=12)
Age at onset, years	20.9±12.2 (2–45)	15.4±9.6 (2–38)	32.0±8.8 (19–45)
Disease course, years	13.2±14.3 (1–55)	16.8±16.1 (1–55)	5.5±3.8 (1–12)
Family history	13/14 (92.9)		
Distal-limb weakness	21 (77.8)	14 (93.3)	7 (58.3)
Tremor	2 (7.4)	2 (13.3)	0
Pes cavus	19 (70.4)	9 (60)	10 (83.3)
Muscular atrophy of lower limbs	21 (77.8)	14 (93.3)	7 (58.3)
Muscular atrophy of upper limbs	13 (48.1)	9 (60)	4 (33.3)
Sensory deficit	13 (48.1)	10 (66.7)	3 (25.0)
CNS involvement	6 (22.2)	4 (26.7)	2 (16.7)
Electrophysiological data	**Total (n=20)**	**Males (n=11)**	**Females (n=9)**
CMTNS	10.6±4.6 (2–18)	12.4±3.5 (4–18)	8.4±4.8 (2–18)
MNCV			
Median CMAP, mV	2.8±1.8 (0.3–7.2)	2.5±2.1 (0.3–7.2)	3.0±1.2 (1.1–4.9)
Median MNCV, m/s	37.6±7.6 (27.3–53.6)	36.1±6.9 (27.3–53.2)	39.5±7.9 (30.8–53.6)
Ulnar CMAP, mV	3.1±1.7 (1.2–8.5)	2.5±1.0 (1.3–4.3)	3.9±2.1 (1.2–8.5)
Ulnar MNCV, m/s	40.0±6.8 (28.9–52.9)	36.8±6.1 (28.9–52.9)	44.0±5.3 (33.1–51.3)
SNCV			
Median SNAP, mV	3.8±3.5 (0.4–15.2)	3.2±2.7 (0.4–9.4)	4.3±4.2 (1.3–15.2)
Median SNCV, m/s	38.7±4.1 (32.1–47.6)	38.1±3.6 (32.1–44.4)	39.5±4.5 (34.5–47.6)
Ulnar SNAP, mV	2.8±1.7 (0.3–7.2)	2.8±1.4 (1.4–5.4)	2.9±2.2 (0.3–7.2)
Ulnar SNCV, m/s	38.3±4.7 (30.3–49.3)	37.2±3.7 (30.3–44.0)	40.2±5.4 (32.5–49.3)

Data are mean±standard deviation (range) or n (%) values.
CMAP, compound muscle action potential; CMTNS, Charcot-Marie-Tooth neuropathy score version 2; CNS, central nervous system; MNCV, motor nerve conduction velocity; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity.

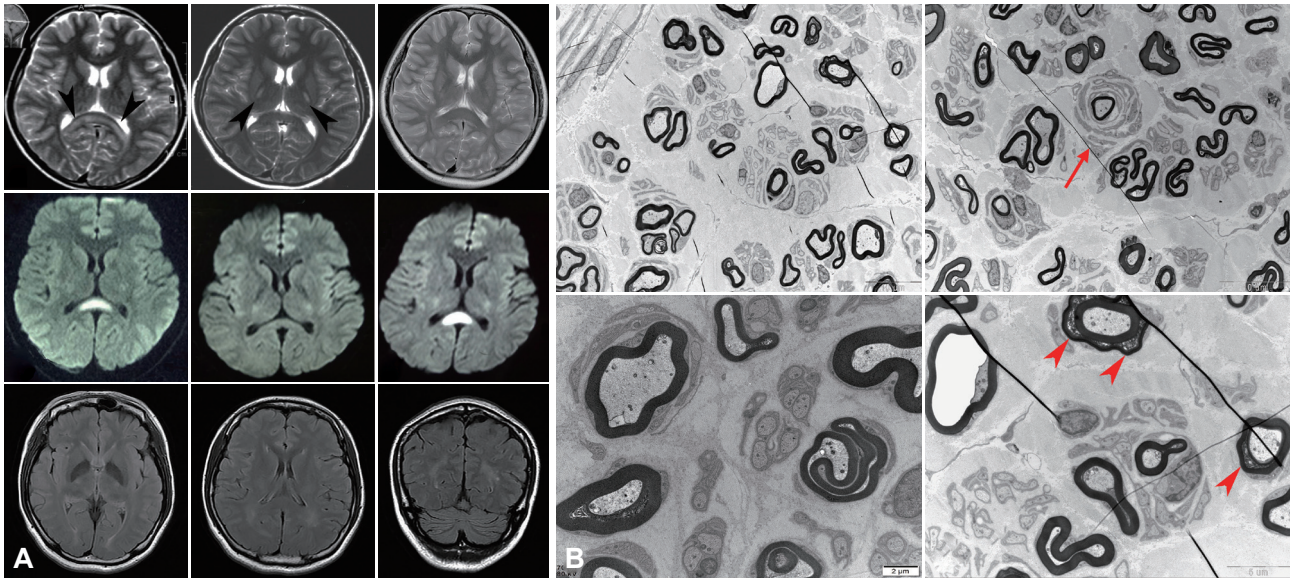

Fig. 1. Brain imaging and neuropathological characterizations of patients with X-linked Charcot-Marie-Tooth disease type 1. A: Brain MRI findings. Upper line (patient F4–II:1): Abnormal signals in the splenium of the corpus callosum and posterior limbs of the bilateral internal capsule (1st, 2nd image) in T2-weighted imaging, while the lesions significantly improved after 6 months (3rd image). Middle line (patient F10–III:6): Abnormal signals in the splenium of the corpus callosum were observed in diffusion-weighted imaging performed 3 years previously (1st image), and the lesions had completely resolved 2 months later (2nd image). These lesions reappeared at the time of the present examinations (3rd image). Lower image (patient F13–III:1): White-matter hyperintensities in the bilateral frontal and parietal lobes, and in the right insular cortex on T2-weighted fluid-attenuated inversion-recovery imaging. B: Neuropathological findings. Upper line: Moderate loss of large myelinated fibers and an increasing number of regenerated axon clusters with abnormally thin myelin sheaths; the onion-bulb formation in patient F13–III:1 is indicated by an arrow. Lower line: Thickened and excessively folded myelin (left image) was observed in patient F9–II:2, and enlargement and widening of the Schmidt-Lanterman incisures (right image, arrowheads) were observed in patient F13–III:1.

by mouth-angle deflection, frequent blinking, and an inability to speak clearly. These episodes were always brief, lasting about 1 minute. The attacks occurred once or twice each month and could be completely relieved by taking carbamazepine (800 mg/day). Brain MRI revealed a cyst and mild atrophy in the cerebellum. Patient F10–III:6, a 30-year-old male, was first referred to our emergency room due to the recurrent paroxysmal dizziness, without motor aphasia, dysphagia, hemiplegia, or quadriplegia, which often lasted for hours. Brain MRI performed 3 years previously had revealed an abnormal signal in the splenium of the corpus callosum in diffusion-weighted imaging, while the lesions were completely resolved 2 months later (Fig. 1A, middle line, 1st and 2nd image). We ascribed this to periphery neuropathy only after we observed weakness of the distal lower limbs, tendon areflexia in all extremities, and pes cavus in a physical examination. Minor foot deformities were detected through detailed medical history tracking since the age of 15 years but they were not taken seriously. An electrophysiological examination revealed slowing of the nerve conduction velocity (NCV) (range, 29.6–38.5 m/s) and reduced action potential amplitudes in both the motor and sensory nerves. The lesions in the splenium of the corpus callosum reappeared at the time of this examination (Fig. 1A, middle line, 3rd image). Patient F13–III:1 had no clinical signs of CNS dysfunction, and white-matter hyperintensities were observed in the bilateral frontal and parietal lobes and the right insular cortex (Fig. 1A, lower line).

Neuropathological findings

A sural nerve biopsy was performed on several probands. Electron micrographs in the transverse sections of the sural nerves of patients with CMTX1 revealed the moderate loss of large myelinated fibers and an increased number of regenerated axon clusters with abnormally thin myelin sheaths (Fig. 1B, upper line). Typical onion-bulb-like structures were observed (Fig. 1B, upper line, right image). Additionally, thickened and excessively folded myelin was occasionally observed (Fig. 1B, lower line, left image). Ultrastructural studies revealed enlargement and widening of the Schmidt-Lanterman incisures (Fig. 1B, lower line, right image), and the appearance of dense granules and vacuolated aggregates in several axons.

Mutation analysis of *GJB1*

We identified 14 diverse *GJB1* variants among 27 patients with CMTX1: 10 missense, 2 frameshift insertion/deletion, 1 nonsense, and 1 synonymous mutations. Eight of these variants had been identified previously, and the remaining six

were novel mutations: c.9G>A (p.Trp3*), c.29T>C (p.Leu-10Pro), c.59T>C (p.Ile20Thr), c.207C>A (p.Phe69Leu), c.403_404insT (p.Tyr135Leufs*12), and c.428_430delTGT (p.Leu143del) (Fig. 2). All of the novel mutations were highly conserved in multiple species and were absent from the 1,000 Genomes Project, dbSNP, and gnomAD databases, as well as from 200 healthy controls. The frameshift insertion-deletion mutation resulted in prematurely truncated proteins, and the deletion of a trinucleotide TGT induced the loss of leucine at codon 143, which would be expected to cause

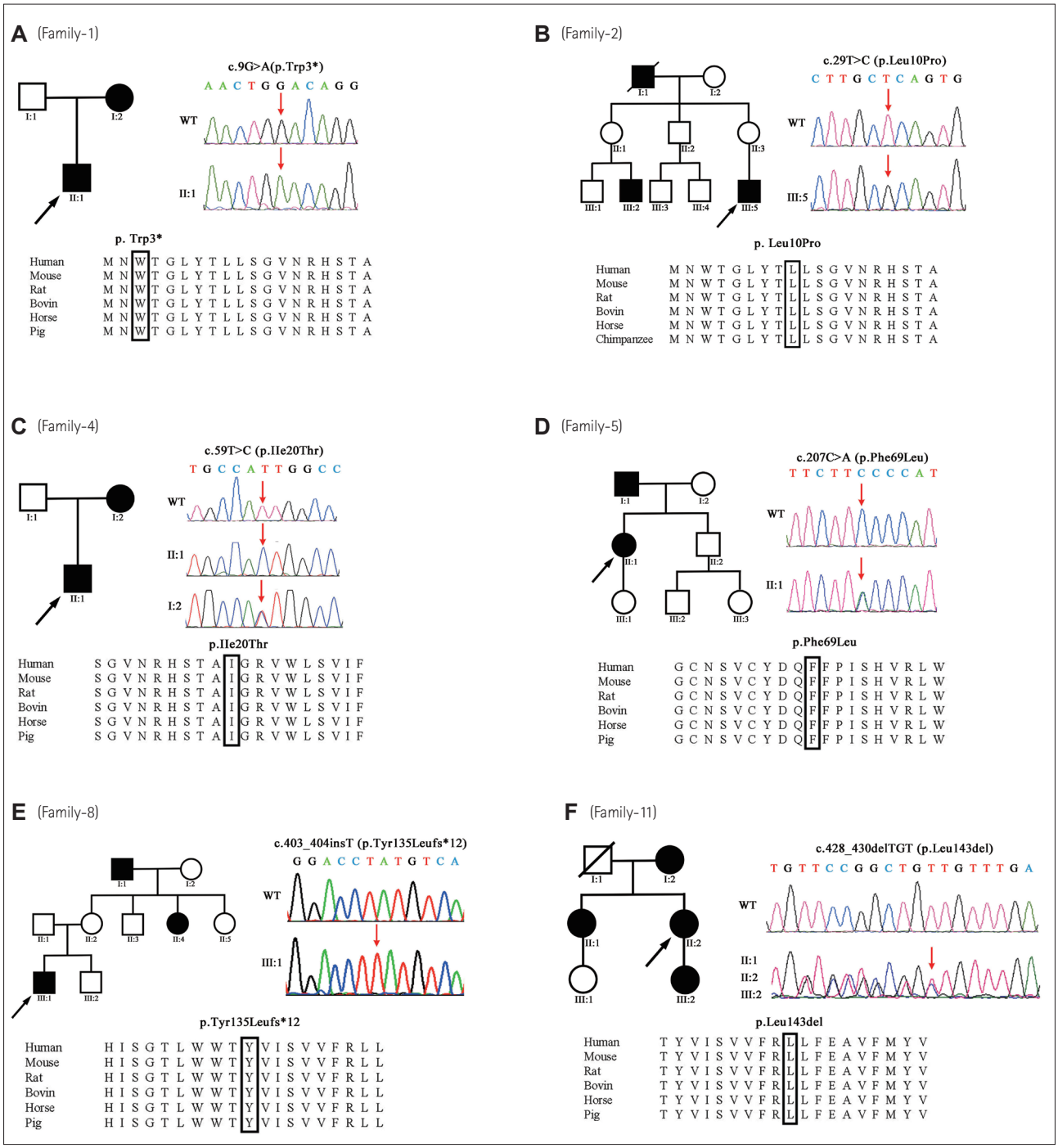

Fig. 2. Pedigrees of Charcot-Marie-Tooth disease type 1 families with novel gap-junction beta-1 mutations. The pedigrees are shown at the top left, the sequencing chromatograms are shown at the top right, and the mutations located in the highly conserved protein region are shown at the bottom. The affected individuals and unaffected family members are indicated by black and open symbols, respectively. The probands are marked by arrows. A: Family-1. B: Family-2. C: Family-4. D: Family-5. E: Family-8. F: Family-11. WT, wild type.

a conformation rearrangement of the protein structure. Based on the prediction results of in silico analyses, all six of these novel mutations were predicted with high probability to be pathogenic (Supplementary Table 2 in the online-only Data Supplement). Except for only one mutation with uncertain significance (c.30C<T), all of the remaining ones were therefore classified as likely pathogenic or pathogenic according to the ACMG guidelines (Supplementary Table 1 in the online-only Data Supplement).

DISCUSSION

This study identified 14 different *GJB1* mutations, including 6 novel ones. We analyzed the clinical, electrophysiological, and neuropathological findings of patients with *GJB1* variants, and affirmed the previous findings that male patients present at a younger age and with more-severe phenotypes than do females.[12-15] The clinical onset of CMTX1 in most affected males ranged from early childhood to the second decade. Gait instability, difficulty running, frequently sprained ankles, and foot deformities were the most common initial presentations. The symptoms in the hands can gradually develop over time to varying degrees. Muscle atrophy (particularly of the intrinsic hand muscles), paresthesia, and sensory loss may be more prominent in patients with CMTX1.[16] The moderate reduction of NCVs and reduced CMAP in most of our patients conformed to the typical intermediate electrophysiological features of CMTX1, suggesting a mixed axonal and demyelinating underlying pathology. Moreover, electrophysiological evidence of distally accentuated axonal damage has been found in patients with CMTX1: the peroneal and tibial motor responses are often absent, the median and ulnar motor responses are often reduced, and electromyography can confirm the length-dependent loss of motor units.[9] Similarly, nerve biopsies of patients with CMTX1 in our cohort also disclosed a mixed neuropathy. Onion-bulb formations were prominent in some cases, but were seldom well-developed. In particular, thickening and outfolding of the myelin sheath were also very rare, despite these features being the striking pathological hallmarks of some CMT4 subtypes.[17,18] The genotype and phenotype correlation of CMT1X is actually weak, and most *GJB1* mutations appear to produce a similar phenotype. We found variability among the affected males, even those from the same family, suggesting that epigenetic factors modify the disease severity.

In addition to typical peripheral neuropathy, many patients with *GJB1* mutations appear to be also present with clinical, neurophysiological, and/or neuroimaging findings of CNS involvement. The characteristic features of episodic CNS dysfunction and abnormal white-matter signals on MRI are instructive for the clinical and genetic diagnosis of CMTX.[8] Six of the patients in our cohort had CNS manifestations in the form of symptoms, signs, and/or neuroimaging that were not associated with the stage or severity of peripheral neuropathy. The paroxysmal symptoms included episodic dysphasia, dizziness, and epileptic seizures, and usually resolved between from a few minutes to hours. Only one patient (F4−II:1) had a precipitating factor such as cold or fever. Other suggested triggering factors include high altitude, hyperventilation, intense exercise, and concussion.[7,19] Subclinical evidence of CNS involvement was also illustrated by abnormal signs (horizontal nystagmus and gait ataxia) (F8−III:1), prolonged latencies of brainstem auditory evoked potentials (F7−III:3), and punctate hyperintensity in white matter (F13−III:1). More interestingly, transient and reversible white-matter lesions were also identified. Patient F10−III:6 visited the clinic due to paroxysmal dizziness with white-matter lesions, while a detailed physical examination unexpectedly revealed the possibility of peripheral neuropathy; further clinical and genetic analyses confirmed the CMTX1 diagnosis. Due to the CNS involvement in CMTX1, its severity ranges from static and subclinical to episodic and florid, which reminded us of the importance of performing a detailed assessment of the family history and a neurological examination. Several CNS phenotypes have been commonly described among previous CMTX1 cases: 1) subclinical abnormalities of evoked potentials, such as visual, auditory, motor, and sensory,[8] 2) positive CNS examination signs or neuroimaging with subtle clinical manifestations, and 3) transient or persistent CNS dysfunction accompanied by white-matter lesions. The CNS manifestation is a variant combination of dysarthria, dysphagia, hemiparesis or tetraparesis, ataxia, cranial nerve deficits, aphasia, diplopia, vertigo, and dyspnea. Moreover, acute disseminated encephalomyelitis-like attacks have also been found.[20] Regarding brain MRI, symmetrical diffuse white-matter hyperintensities often involve the centrum semiovale, periventricular, and splenium or genu of the corpus callosum.[7] Diffuse involvement of the corticospinal or corticobulbar tracts has also been described.[21] Reversible MRI abnormalities usually improve within from 1 months to 2 years. CMTX1 should also be included as a differential diagnosis for patients with other episodic neurological diseases, as well as those with peripheral neuropathy and reversible cerebral white-matter degeneration.[19] An electrophysiological examination has been suggested as helpful, with the median MNCV being a sensitive and valuable parameter.[22]

GJB1 encodes for Cx32, which constitutes gap-junction channels that facilitate direct diffusional exchange between neighboring cells, which in turn modulates and synchronizes their intracellular environments.[9,23] Cx32 is widely ex-

pressed by many cell types, including Schwann cells and oligodendrocytes.[8,24] It is also highly conserved and homologous, and has four transmembrane (TM) domains with an alpha-helix structure, and one intracellular and two extracellular loops with intracellular N- and C-terminal domains. All of these domains play a large role in channel properties.[9,25] Wild-type Cx32 was mostly localized at the intercellular boundaries, whereas mutants presented aberrant cytoplasmic retention.[13,26,27] Cx32-knockout mice developed a progressive demyelinating neuropathy that began at about 3 months old, as well as subtle CNS myelin defects.[28,29] Altered synthesis and/or abnormal channel properties leading to loss of gap-junction function are therefore probably the main abnormalities of mutant Cx32. Moreover, toxic gain-of-function and/or dominant-negative effects have also been demonstrated in very few mutants.[30,31] We identified 14 *GJB1* variants, 8 of which were predicted to be located in the TM domains, suggesting that the affected residues are crucial to the normal function of Cx32. The previously observed synonymous mutation (c.30C>T/p.Leu10Leu)[32] in one of our patients (F3−III:3) presented with marked peripheral neuropathy was classified as a variant with uncertain significance. The novel nonsense (p.Trp3*) and missense (p.Phe69Leu) variants have been found to cause the same amino acid changes with different base substitutions.[12,26] Localized in the alpha helix of the third TM region, the novel frameshift (p.Tyr135Leufs*12) and in-frame (p.Leu143del) indels are predicted to cause disease by affecting protein structure and channel function.[16] Whether the above mutants impair Cx32 function requires further confirmation through functional research. Moreover, the exact etiology of transient and reversible CNS dysfunction of CMTX1 remains ambiguous. A previous study found that vascular endothelial cells also expressed Cx32, which participated in endothelial gap-junction intercellular communication.[33] Cx32 also protected endothelial cells from vascular inflammation by modulating the inflammatory cytokine expression.[34] It is unknown whether immune or inflammation factors are involved in the development of demyelinating neuropathy in CMTX1, but the exposure of myelin and/or Schwann-cell antigens to which central tolerance has not been induced may trigger an autoimmune reaction against myelin in the CNS.[35,36] In short, the molecular mechanism of neuropathy or CNS dysfunction in CMT1X requires further exploration to achieve a therapeutic breakthrough.

In conclusions, this study assessed CMTX1 with episodic CNS dysfunction using detailed clinical, electrophysiological, and neuropathological investigations. Our findings have provided new insight into the phenotypic and genetic spectrum of CMTX1. Although CMTX1 was found to have large phenotypic and CNS involvement variabilities, detailed neurological examinations and nerve conduction studies will provide critical clues for accurate diagnoses. Identification of the underlying mechanisms of Cx32 in the development of neuropathy or CNS dysfunction may hold promise for the development of precise therapeutic strategies.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.3988/jcn.2023.0104.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

ORCID iDs

Feixia Zhan	https://orcid.org/0000-0003-1880-8481
Wotu Tian	https://orcid.org/0000-0003-3170-4046
Yuwen Cao	https://orcid.org/0009-0002-8461-0203
Jingying Wu	https://orcid.org/0000-0003-4582-9966
Ruilong Ni	https://orcid.org/0000-0002-9630-015X
Taotao Liu	https://orcid.org/0000-0003-1517-1622
Yun Yuan	https://orcid.org/0000-0003-3282-9123
Xinghua Luan	https://orcid.org/0000-0001-6683-5105
Li Cao	https://orcid.org/0000-0003-1742-9877

Author Contributions

Conceptualization: Li Cao. Data curation: all authors. Funding acquisition: Li Cao. Investigation: Feixia Zhan, Wotu Tian, Xinghua Luan, Li Cao. Methodology: Feixia Zhan, Wotu Tian, Yun Yuan, Xinghua Luan, Li Cao. Project administration: Li Cao. Supervision: Xinghua Luan, Li Cao. Validation: Feixia Zhan, Li Cao. Visualization: Xinghua Luan, Li Cao. Writing—original draft: Feixia Zhan. Writing—review & editing: Feixia Zhan, Xinghua Luan, Li Cao.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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