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CASE REPORT

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Hereditary spastic paraplegia and extensive leukoencephalopathy: a case report of a unique phenotype associated with a *GJB1*/Cx32 p.Pro174Ser variant

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Abstract

Background Pathogenic variants in Gap junction protein beta 1 (*GJB1*), which encodes Connexin 32, are known to cause X-linked Charcot-Marie-Tooth disease (CMTX), the second most common form of CMT. CMTX presents with the following five central nervous systems (CNS) phenotypes: subclinical electrophysiological abnormalities, mild fixed abnormalities on neurological examination and/or imaging, transient CNS dysfunction, cognitive impairment, and persistent CNS manifestations.

Case presentation A 40-year-old Japanese male showed CNS symptoms, including nystagmus, prominent spastic paraplegia, and mild cerebellar ataxia, accompanied by subclinical peripheral neuropathy. Brain magnetic resonance imaging revealed hyperintensities in diffusion-weighted images of the white matter, particularly along the pyramidal tract, which had persisted since childhood. Nerve conduction assessment showed a mild decrease in motor conduction velocity, and auditory brainstem responses beyond wave II were absent. Peripheral and central conduction times in somatosensory evoked potentials elicited by stimulation of the median nerve were prolonged. Genetic analysis identified a hemizygous *GJB1* variant, NM_000166.6:c.520C > T p.Pro174Ser.

Conclusions The patient in the case described here, with a *GJB1* p.Pro174Ser variant, presented with a unique CNS-dominant phenotype, characterized by spastic paraplegia and persistent extensive leukoencephalopathy, rather than CMTX. Similar phenotypes have also been observed in patients with *GJC2* and *CLCN2* variants, likely because of the common function of these genes in regulating ion and water balance, which is essential for maintaining white matter function. CMTX should be considered within the spectrum of *GJB1*-related disorders, which can include patients with predominant CNS symptoms, some of which can potentially be classified as a new type of spastic paraplegia.

Keywords X-linked Charcot-Marie-Tooth disease, *GJB1*, Spastic paraplegia, Leukoencephalopathy

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Background

Pathogenic variants in Gap junction protein, beta 1 (*GJB1*), a gap junction family gene located at Xq13.1 cause X-linked Charcot-Marie-Tooth disease (CMTX) [1]. Connexin 32 (Cx32), encoded by *GJB1*, is expressed in the myelinating Schwann cells of peripheral nerves, which are primarily affected in CMTX. Cx32 is also widely localized in outer oligodendrocyte membranes in the central nervous system (CNS) [2]. Reflecting this, five CNS phenotypes are recognized in CMTX [3]: (1) subclinical abnormalities of visual- and auditory-evoked responses, (2) overt mild fixed abnormalities on neurological examination and/or CNS imaging that may or may not be accompanied by clinical manifestations, (3) severe transient CNS dysfunction accompanied by white matter changes observed by magnetic resonance imaging (MRI), (4) mild to severe cognitive impairment, and (5) persistent central nervous manifestations. The third phenotype, of severe, transient CNS symptoms, such as aphasia, dysarthria, ataxia, monoparesis, hemiparesis, paraparesis, or tetraparesis [2, 4], lasting from hours to weeks, is particularly well documented. The second and fifth phenotypes include persistent symptoms that are often associated with mild, persistent abnormalities on CNS imaging [5–7].

Here, we report a Japanese patient with the *GJB1* variant, NM_000166.6:c.520C > T p.Pro174Ser, characterized by spastic paraplegia associated with persistent and extensive leukoencephalopathy involving the pyramidal tracts. Clinically, this patient could potentially be diagnosed with hereditary spastic paraplegia rather than CMTX. Notably, this case features long-term MRI follow-up over 16 years and the first electrophysiological results associated with this *GJB1* variant. We also present positive MRI and electrophysiological findings in a female carrier of this variant. The phenotype observed in this patient is similar to those seen in patients with variants of *GJC2* and *CLCN2*, suggesting that these genes and *GJB1* contribute to a common phenotype through their role in regulating ion and water homeostasis in the brain [8].

Case presentation

The patient was a 40-year-old male (Fig. 1A). His birth was unremarkable. He began rolling over at 4 months and was able to crawl by 8 months. By age 2, he had not yet started walking, and during a health check-up at that age, a pediatrician noted delayed motor development and gaze-evoked horizontal nystagmus. He experienced several febrile convulsions starting at age 1 and began anti-epileptic medications at age 4. At that time, a pediatric neurologist observed nystagmus, ataxia, and lower limb spasticity. Although he was able to walk unaided during his teenage years, his trunk balance remained unstable. His motor function progressively worsened, leading him to use a walking stick by age 28 and a wheelchair for outdoor activities. MRI revealed increased T2 signal in the white matter. At age 30, anti-epileptic medications were discontinued after 26 years of remission. At age 40, during a clinical examination at our hospital, the patient exhibited slight impairments in retrograde memory, visuospatial ability, motor programming, sensitivity to interference, and word retrieval, as indicated by his scores on several cognitive assessments: Montreal Cognitive Assessment [9] (21/30, with specific deficits in visuospatial ability: -3, attention: -2, language: -2. abstraction: -1, and retrograde memory: -1), Revised Hasegawa Dementia Scale [10] (30/30), Addenbrooke's Cognitive Examination-Revised [11] [96/100, with specific deficits in retrograde memory: -2 and visuospatial ability (clock drawing): -2], and Frontal Assessment Battery [12] (16/18, with specific deficits in motor programming: -1 and sensitivity to interference: -1). He displayed impaired smooth pursuit eye movement and gaze-evoked horizontal nystagmus. Muscle tone in the lower extremities showed marked spasticity. Although there was pes equinovarus in the lower extremities, muscle atrophy was minimal or very mild (Fig. 1B). Manual muscle test scores (right/left) revealed a pyramidal pattern of weakness in the lower limbs: iliopsoas 4-/3+, quadriceps 5-/5-, hamstrings 3/3, tibialis anterior 2/2, gastrocnemius 2/2, toe extension 1/1, and toe flexion 4/4. Muscle strength in the upper limbs remained preserved. Tendon reflexes in the lower limbs were markedly increased, with positive Babinski sign and Chaddock pathological reflexes, while those in the upper limbs were normal. No abnormalities were noted in the sensory nervous system, including touch, pain, vibration, and position senses. The finger-to-nose and heel-to-knee test demonstrated mild cerebellar ataxia. Laboratory tests showed normal levels of creatinine kinase, lactate, pyruvic acid, and thyroid hormones, along with a negative result for anti-HTLV1 antibody. Anti-HIV antibody, fluoride, and very long-chain fatty acid levels were not tested. Cerebral spinal fluid analysis revealed a slightly elevated protein level (57 mg/dl) without pleocytosis. Brain MRI by T2 fluid-attenuated inversion recovery and diffusion-weighted imaging showed diffuse hyperintensity in the white matter, particularly along the pyramidal tract from the posterior limb of the internal capsule to the cerebral peduncle, with additional involvement in the occipital lobe and cerebellar peduncles, medial lemniscus, and corpus callosum (Fig. 1D). Mildly low apparent diffusion coefficient values were present in the same lesion (Fig. 1D), indicating myelin

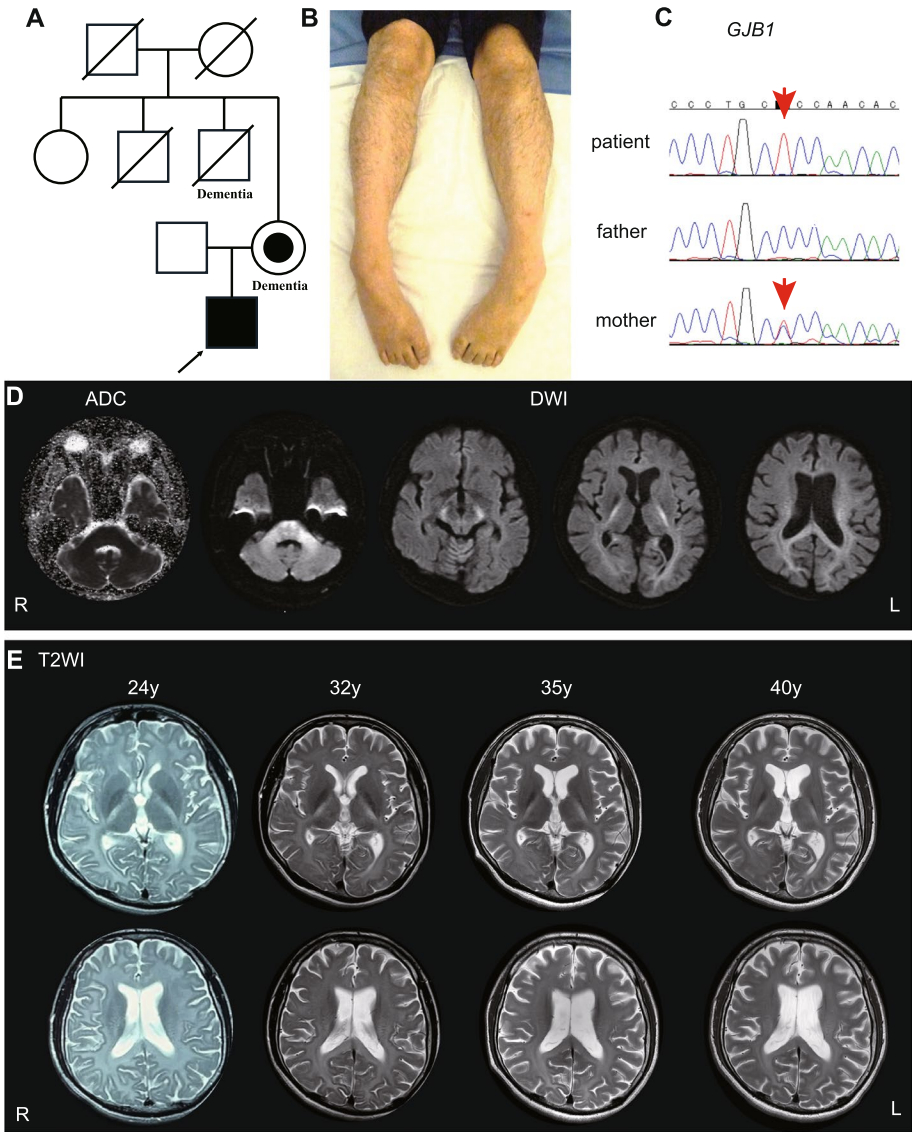

Fig. 1 **A** Family pedigree of the patients in the present case. **B** Pes equinovarus and minimal atrophy of the lower limbs. **C** Sanger sequencing of the *GJB1*:(c.520C > T, p.Pro174Ser) variant in family members. Arrows indicate the position of the variant. **D** Apparent diffusion coefficient (ADC) map and diffusion-weighted images (DWI) of the brain of the paatient acquired at age 40. **E** Temporal change of T2-weighted imaging (T2WI) in the patient from age 24 to 40

microvacuolation instead of hypomyelination [13]. These signal abnormalities have remained stable from age 24 to 40 (Fig. 1E). Nerve conduction assessment indicated a moderate decrease in sensory nerve action potentials of the median, ulnar, and sural nerves, and a mild decrease in motor conduction velocities of the median, ulnar and tibial nerves (36–44 m/sec), indicating subclinical polyneuropathy (Table 1A). Electroencephalography yielded normal results, while brainstem auditory-evoked responses beyond wave II were absent. Prolonged peripheral and central conduction times were observed in somatosensory evoked potentials elicited by left median nerve stimulation (N9o-P13/14o latency: 6.4 ms; P13/14o-N20o latency: 9.4 ms).

Given the patient's predominant symptoms of spastic paraplegia, early onset, and gradual progression, exome sequencing was performed for both the patient and his parents. This analysis identified a hemizygous missense variant in *GJB1* (NM_000166.6:c.520C > T p.Pro174Ser) in the patient and heterozygosity for this variant in his mother, which were confirmed by Sanger sequencing (Fig. 1C). In accordance with the guidelines from the

Table 1 Nerve conduction studies of the patient (A) and the mother (B)

A Motor nerves				
Nerve		Latency (ms)	Amplitude (mV)	Conduction velocity (m/s)
Median	Wrist	4.3	11.6	
	Elbow	9.8	11.1	36.0
Ulnar	Wrist	3.2	16.6	
	Below elbow	7.9	16.1	40.4
	Above elbow	9.3	15.4	35.7
Tibial	Ankle	3.4	11.4	
	Knee	11.7	6.7	37.3
Fibular	Ankle	5.2	0.8	
	Below knee	14.2	1.0	36.1
	Knee	15.5	1.0	50.0
Orthodromic sensory nerves				
Nerve		Latency (ms)	Amplitude (μV)	Conduction velocity (m/s)
Median		2.8	1.8	44.2
Ulnar		2.7	2.9	38.9
Sural		4.2	1.4	35.9
B Motor nerves				
Nerve		Latency (ms)	Amplitude (mV)	Conduction velocity (m/s)
Median	Wrist	5.4	4.67	
	Elbow	9.5	3.62	41.5
Ulnar	Wrist	2.9	13.82	
	Below elbow	6.3	12.64	54.4
	Above elbow	7.8	12.55	33.3
Tibial	Ankle	3.9	16.25	
	Knee	12.5	9.79	40.1
Fibular	Ankle	6.2	0.4	
	Below knee	13.8	0.51	37.5
	Knee	16.5	0.55	31.5
Orthodromic sensory nerves				
Nerve	Latency (ms)	Amplitude (μV)	Conduction velocity (m/s)	
Median	3.2	5.9	37.5	
Ulnar	2.12	5.1	47.2	
Sural	4.32	0.7	34.7	

Nerve conduction studies were performed on the left side median, ulnar, tibial, fibular, and sural nerves

American College of Medical Genetics and Genomics [14], this variant was classified as pathogenic (PS1 + PM2 + PP1 + PP2 + PP3 + PP4).

His 73-year-old mother (Fig. 1A) also presented with subclinical neuropathy, as evidenced by a mild decrease in nerve conduction velocity, and mild abnormal intensity in corticospinal tract diffusion-weighted images (Fig. 2A, Table 1B). She exhibited severe sensory aphasia with semantic jargon attributable to atrophy and decreased blood flow in the left temporal lobe, extending from the temporal pole to the temporoparietal region (Fig. 2B). On the Revised Hasegawa Dementia Scale, she was unable to understand the meaning of tasks and scored only 1/30, comprehending only her age. No known variants associated with semantic dementia were identified in the mother by exome sequencing.

Discussion and conclusions

The patient in our case, who carries a *GJB1* variant that is primarily recognized as a causative gene for CMTX, is notable for the predominance of progressive spastic paraplegia, minimal peripheral nervous system (PNS) involvement, and the presence of persistent extensive white matter abnormalities, including those affecting the corticospinal tract. Clinically, hereditary spastic paraplegia was strongly suspected over CMT, while radiologically, leukoencephalopathy was a notable consideration. Indeed, a previously reported case with the

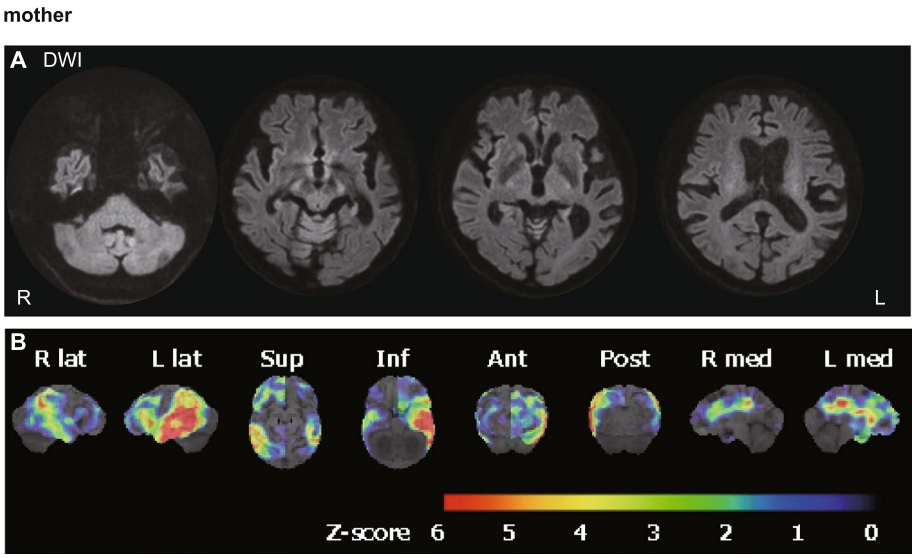

Fig. 2 **A** diffusion-weighted images (DWI) of the patient's mother's brain. **B** ^{123}I-N-isopropyl-p-iodoamphetamine single-photon emission computed tomography (SPECT) imaging of the mother. The Z-score maps displayed on an anatomically standardized MRI template are shown

GJB1 p.Pro174Ser variant was described within the context of leukoencephalopathy, not CMT [8]. Their clinical features closely mirror those observed in our case (Table 2). The p.Pro174Ser variant may therefore represent a distinct phenotype characterized by spastic paraplegia and persistent extensive white matter abnormalities involving the pyramidal tract and middle cerebellar peduncles. The 16-year MRI follow-up of the patient provided clear evidence of the persistence of white matter abnormalities. Additionally, while a previous study had not observed MRI abnormalities in female carriers of this gene variant [8], the mother of the present patient exhibited notable findings, including mild MRI changes in the pyramidal tract and reduced nerve conduction velocities. The differences in phenotypes among female carriers may be partially explained by the biased pattern of X-chromosome inactivation in individual myelinating glial cells [15].

The reason for only certain *GJB1* variants resulting in persistent CNS symptoms that significantly deviate from the typical CMT phenotype remain unclear. An analysis of structural domains in Cx32 variants

Table 2 Clinical manifestations of patients with the *GJB1* p.Pro174Ser variant

	Patient	***Patient's mother***	***Depienne* et al., *2013***
Sex/ancestry	male/Asian	female/Asian	male/European
Consanguineous parents	No	No	No
Affected family members	Yes	Yes	Yes
Early psychomotor development			Normal, but has never walked without support
Age at first sign	1 year and 9 months		12 months
Disease course	Slowly progressive		Slowly progressive
Motor development	delay	normal	delay
Age requiring wheelchair use	28	independent	N/A
Cognitive level	Mild, word-finding difficulties	Severely impaired	Mild learning disability
Nystagmus	Yes	No	Yes
Hearing	Normal	Normal	Normal
Spasticity	Yes, prominent	No	Yes, prominent
Ataxia	Yes	No	Yes, severe
Peripheral polyneuropathy	No clinical signs: mildly decreased motor NCV	No clinical signs: mildly decreased motor NCV	No clinical signs: mildly decreased motor NCV

indicates a genotype–phenotype correlation in CMTX, with variants in the intracellular cytoplasmic domain showing less severe phenotypes compared with variants in other domains [16]. p.Pro174Ser is located in the second transmembrane domain, but variants associated with chronic corticospinal tract dysfunction, such as p.Ala39Val [17], p.Thr55Ile [18], p.Met93Val [19], p.Arg164Gln [18], p.Arg183His [20], p.Thr191 frameshift [21], and p.Leu143Pro [22], are not situated in the intracellular cytoplasmic domain and do not cluster in any specific domain.

The spastic paraplegia and white matter abnormalities seen in our patient are primary characteristics of several disorders, making differential diagnosis crucial [23]. One such disorder is caused by certain variants of *GJC2*, which encodes Cx47, a gap junction protein family member primarily expressed in oligodendrocytes [24]. The symptoms and imaging findings associated with this variant are similar to those observed in our case [24]. While most pathogenic *GJC2* variants lead to Pelizaeus-Merzbacher-like disease type 1 (PMLD1) or hypomyelinating leukodystrophy 2 (HLD2) [25], a rare type characterized by prominent spastic paraplegia is classified as autosomal recessive spastic paraplegia type 44 (SPG44) [26]. Despite the presence of white matter abnormalities, severe cognitive impairment is rare in SPG44 [24], and was not observed in a patient with the Cx32 p.Pro174Ser variant [8]. Similarly, cognitive function was generally preserved in our patient. The mother of our patient showed severe cognitive decline consistent with semantic dementia, yet her white matter abnormalities were milder than those observed in the patient, indicating no relationship with the *GJB1* p.Pro174Ser variant.

Another example is that loss-of-function *CLCN2C* variants cause similar phenotypes to those observed in our patient [8], suggesting that *GJB1*, *GJC2*, and *CLCN2C* may have related functions. The white matter of the brain is primarily composed of axons with myelin sheaths, and its most crucial physiological function, impulse conduction, depends on the movement of ions and water. A loss-of-function variant of *CLCN2*, which encodes the ClC-2 chloride channel involved in ion and water homeostasis in the brain, can cause leukoencephalopathy [8]. Similarly, Cx32 forms channels between opposing membranes of adjacent cells to create gap junctions between axons and myelinating Schwann cells or oligodendrocytes, which facilitate the movement of small molecules and ions between myelin and axons [19]. Given the channel function of Cx32 and CIC-2, it is reasonable to assume that loss of channel function leads to demyelination, resulting in a common CNS phenotype of spastic paralysis and white matter abnormalities. Similarly, abnormalities in Cx47, which, like Cx32, belongs to the connexin family are thought to produce comparable effects.

By contrast, patients with total *GJB1* deletion show typical CMT1-like symptoms without CNS involvement [27]. In this scenario, the impact of Cx32 loss of function is limited to the PNS, while compensatory mechanisms by other oligodendrocyte gap junction proteins, including Cx47, may operate in the CNS [27]. Considering this, the Cx32 p.Pro174Ser variant is speculated to act in a dominant-negative manner in the CNS to reduce the function of not only Cx32 but all oligodendrocyte gap junction proteins. Conversely, in the PNS, the loss-of-function effect of this variant on Cx32 is estimated to be relatively mild.

Furthermore, although the genetics and pathomechanisms differ, X-linked adrenoleukodystrophy also shows high signal intensity along white matter tracts on MRI and causes demyelination in both the CNS and PNS [28, 29]. Therefore, in hereditary diseases that present with demyelinating lesions, such as in the patient case, it is important to focus on both the CNS and PNS during clinical evaluation.

In some patients with *GJB1* variants, including the case presented here, the clinical diagnosis may be hereditary spastic paraplegia or leukoencephalopathy rather than CMT. Therefore, just as PMLD1/HLD2 and SPG44 are regarded as part of the spectrum of *GJC2*-related neurological disorders [24], CMTX should be recognized as part of the spectrum of *GJB1*-related disorders. This spectrum may include patients with predominant CNS phenotypes, as well as those that could potentially be classified as a novel type of spastic paraplegia.

Abbreviations

CMTX	X-linked Charcot-Marie-Tooth disease
GJB1	Gap Junction Protein, Beta 1
CNS	Central nervous systems
Cx32	Connexin 32
MRI	Magnetic resonance imaging
PNS	Peripheral nervous system
PMLD1	Pelizaeus-Merzbacher-like disease type 1
HLD2	Hypomyelinating leukodystrophy 2
SPG44	Spastic paraplegia type 44

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Authors' contributions

H.N., H.D. and F.T drafted the manuscript; E.T. and Y.M. performed electrophysical analysis; H.F., T.W., A.F., and N.M. identified the GJB1 variant; M.T., Y.H., Y.N., K.K., M.H. and H.K. conducted clinical evaluations. The final manuscript was read and approved by all authors.

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Data availability
No datasets were generated or analysed during the current study.

Availability of data and materials
The DNA sequencing data analyzed during the current study is available in the DNA Data Bank of Japan [Accession number LC822818].

Declarations

Ethics approval and consent to participate
Research protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine (A130530002), and written informed consent was obtained from all patients.

Consent for publication
Written informed consent for publication was obtained from all patients.

Competing interests
The authors declare no competing interests.

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