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Two Cases of Multiple Sclerosis in a Family With X-Linked Charcot-Marie-Tooth Disease

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Abstract

Background and Objectives

The aim of this study was to present a case report of the first familial case of multiple sclerosis (MS) in an X-linked Charcot-Marie-Tooth family with a novel variant in *GJB1*.

Methods

Clinical, neurophysiologic, neuroimaging, and genetic assessments were performed on 9 affected members of a large X-linked Charcot-Marie-Tooth (CMTX) family, including 2 who also developed MS. The 2 family members with CMTX and MS were screened for pathogenic variants in 245 genes associated with MS. We tested 150 independent patients with MS, 48 familial and 102 sporadic for rare pathogenic variants in *GJB1*.

Results

A novel missense pathogenic variant (c.502T > G, p.Cys168Gly) in *GJB1* was detected in a large CMTX family. Two 5th-degree relatives developed typical MS in addition to CMTX. No additional pathogenic genetic variants were identified in 245 MS-associated genes in 2 MS patients with exome sequencing data. Furthermore, *GJB1* pathogenic variants were not found in a cohort of 48 patients with familial and 102 with sporadic MS.

Discussion

This is a novel report of a familial case of MS related to the novel variant in GJB1. Although our report adds additional evidence for the increased risk of MS in carriers of pathogenic variants in GJB1, we demonstrate that genetic variation in GJB1 is not a common risk factor, neither in familial nor sporadic MS.

Introduction

While the frequency of familial cases of multiple sclerosis (MS) is around 12%, there are still no convincing monogenic forms of the disease described. Several genetic risk factors were reported, including human leukocyte antigen, genome-wide association study (GWAS) hits,[1] and genes identified by exome sequencing in familial cases.[2]

Individual case reports, and a cohort study of 70 patients with CMTX suggest that pathogenic variants in *GJB1* might act as a risk factor of MS.[3-7] In that case, it could be expected to observe more family members with MS in the CMTX families, which has not been reported so far. We report the first case of familial MS in the CMTX family with a novel *GJB1* pathogenic variant.

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Glossary

CMAP = compound muscle action potential; **CMTX** = Charcot-Marie-Tooth disease, X-linked type; **DMT** = disease-modifying treatment; **EDSS** = Expanded Disability Status Scale; **GWAS** = Genome-Wide Association Study; **GJ** = gap junction channels; **MNCV** = Motor nerve conduction velocity; **MS** = multiple sclerosis; **MUAP** = motor unit action potential; **OCB** = oligoclonal bands of IgG; **RRMS** = relapsing-remitting multiple sclerosis; **SNAP** = sensory nerve action potential; **VEP** = visual evoked potential.

Methods

Genetic Analyses

For the exome sequencing analysis, genomic DNA was extracted from peripheral blood using the Chemagic DNA Blood 4k Kit (Revvity, Waltham, MA) and quantified with the Qubit fluorometric assay. DNA was fragmented with the M220 ultrasonicator (Covaris, Woburn, MA), and exome enrichment was performed using the xGen Exome Research Panel v1.0 (IDT). Libraries were sequenced on the Illumina NextSeq 550 platform (Illumina, San Diego, CA) with 150 bp paired-end reads. Data processing followed genome analysis toolkit best practices: Reads were aligned with Burrows-Wheeler Aligner v0.7.2, variants were called with genome analysis toolkit v3.5 and annotated using snpEff v4.3. Pathogenicity was further assessed with dbNSFP v2.0, incorporating both classical (SIFT, PolyPhen-2, and MutationTaster) and meta-predictors (MetaSVM, combined annotation dependent depletion, and rare exome variant ensemble learner). An in-house variant interpretation software was used for data interpretation and clinical assessment of the sequencing results.

In the exome sequencing data of the 2 patients with MS in the reported family, we have also investigated 245 genes reported to be associated with MS, which included 168 identified in GWAS studies and 77 genes identified by exome sequencing in studies familial MS. The first subset included genes associated with MS based on the GWAS study data (for genes with associations that reached a genome-wide significance value below $1{\cdot}10^{-8}$ in the study of international MS consortium[1]) and the second set included genes that have been associated with MS in exome sequencing studies or represent monogenic causes of diseases presenting with autoinflammation or demyelination in the CNS. The lists of genes are provided in the Supplementary information.

The GJB1 variant identified is referenced in the matched annotation from NCBI and EMBL-EBI select RefSeq transcript NM_000166.6.

Standard Protocol Approvals, Registrations, and Patient Consents

Participation of the subjects in the study was voluntary and anonymous. For each case, a signed consent form was obtained from the patient. The study was conducted in accordance with the principles of the Declaration of Helsinki on biomedical research involving humans and the provisions of the Council of Europe Convention on the protection of human rights and the dignity of the human being, in line with the Oviedo Convention. The study of hereditary factors in MS was approved by the National Ethics Committee (approval number: 90/08/12). For this case report, the CAse REport checklist was followed to ensure methodological quality.

Data Availability

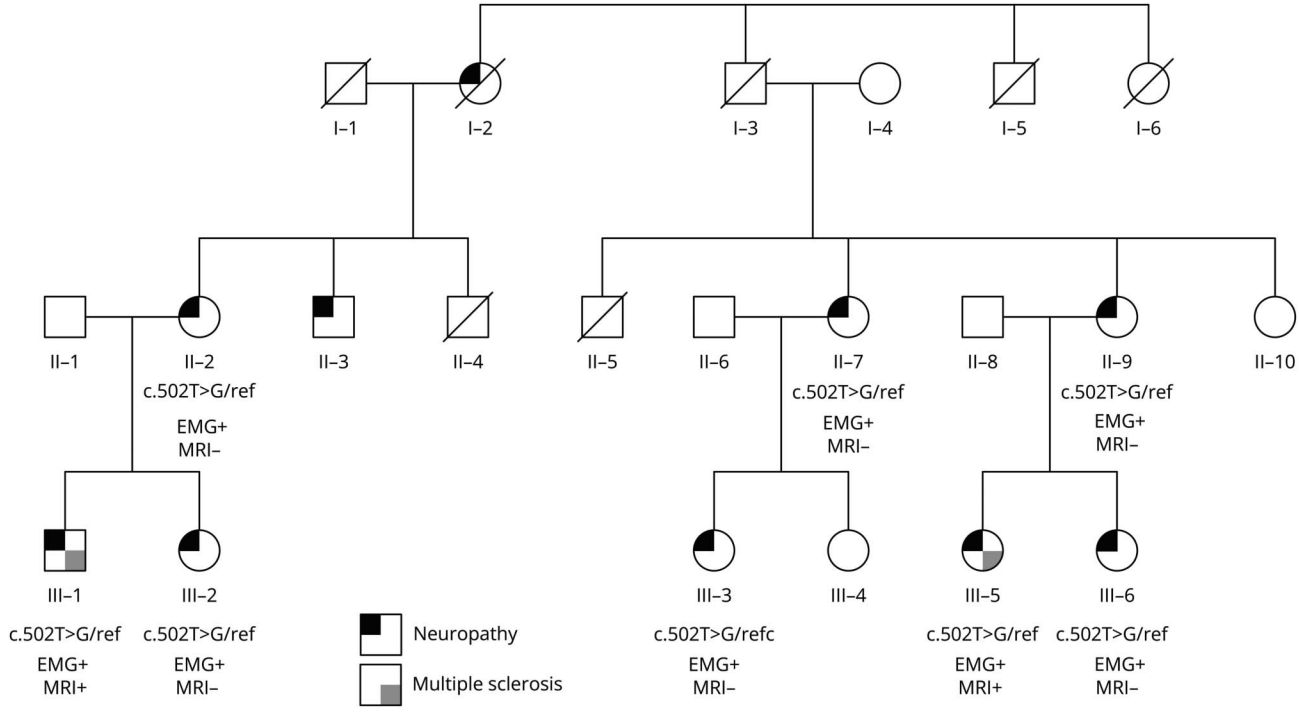
The data on the identified genetic variant was submitted to ClinVar. The full exome sequencing data sets were generated during the routine diagnostic process, and the patients did not consent to release full data sets to public repositories.

Results

We evaluated a Slovene CMTX family with 9 affected family members with neuropathy (Figure 1). Exome sequencing revealed a novel missense pathogenic variant (c.502T > G, p.Cys168Gly) in *GJB1*, which segregated in all affected family members.

The propositus (III.1) was at age 20 years when admitted to the emergency department because of double vision attributed to right abducens nerve palsy. At age 30 years, he developed a 4-month lasting burning pain in the left lower limb and a sensory thoracic level. For 2 days, he was experiencing urinary incontinence and vertigo. The clinical investigation revealed exhaustible horizontal nystagmus and partial paresis of right abducens, as well as muscle atrophy in upper and lower limbs distally. He had bilateral pes cavus and hammer toes and could not perform dorsiflexion of feet and toes. Myotatic reflexes of the limbs were absent. He could walk, although with signs of peroneal weakness on both sides. We found hypesthesia below level T6 and hypoalgesia below level T8. With EMG, we did not obtain sensory nerve action potentials (SNAPs) for the median, ulnar, and sural nerves. Motor nerve conduction velocity (MNCV) of the right median nerve was normal; however, the distal latency of the compound muscle action potential (CMAP) was prolonged (7.9 ms), and its amplitude was very low (0.1 mV). The MNCV of the right ulnar nerve was reduced (31 m/second); the distal latency of the CMAP was prolonged (7.9 ms), and the amplitude was very low (0.1 mV). We did not obtain MNCV for either the fibular or tibial nerves, nor F-wave latencies for any peripheral nerves. Needle EMG of the tibialis anterior and gastrocnemius muscles revealed firing of only a single motor unit action potential (MUAP) with an amplitude of 0.5 mV.

Figure 1 Pedigree



Pedigree of the family with X-linked Charcot-Marie-Tooth and multiple sclerosis. The pedigree displays affection with symptoms of neuropathy and multiple sclerosis. The EMG ⊕ and MRI ⊕ notation reflects the abnormal electromyographic and magnetic resonance report, respectively.

Brain MRI demonstrated diffuse symmetrical confluent periventricular and callosal demyelination lesions (Figure 2, A and B). Thoracic spinal cord MRI did not reveal any convincing demyelinating lesions. Cervical spinal cord MRI showed a demyelination lesion at the C3-C4 level. CSF analysis revealed the presence of oligoclonal bands of IgG (OCB) and intrathecal IgG synthesis. Visual evoked potentials (VEPs) were pathologic and consistent with demyelination. Symptoms of CNS recovered after short-term, high-dose methylprednisolone treatment. According to the 2010 McDonald criteria,[8] relapsing-remitting multiple sclerosis (RRMS) was confirmed. The diagnosis was made on clinical grounds (2 relapses) and paraclinical laboratory assessments emphasizing the dissemination in space (demonstrated by T2 lesion on MRI in 4 locations considered characteristic for MS and as specified (juxtacortical, periventricular, infratentorial, and spinal cord)). The positive CSF findings (2 or more oligoclonal bands) also support the inflammatory demyelinating nature of the underlying condition.

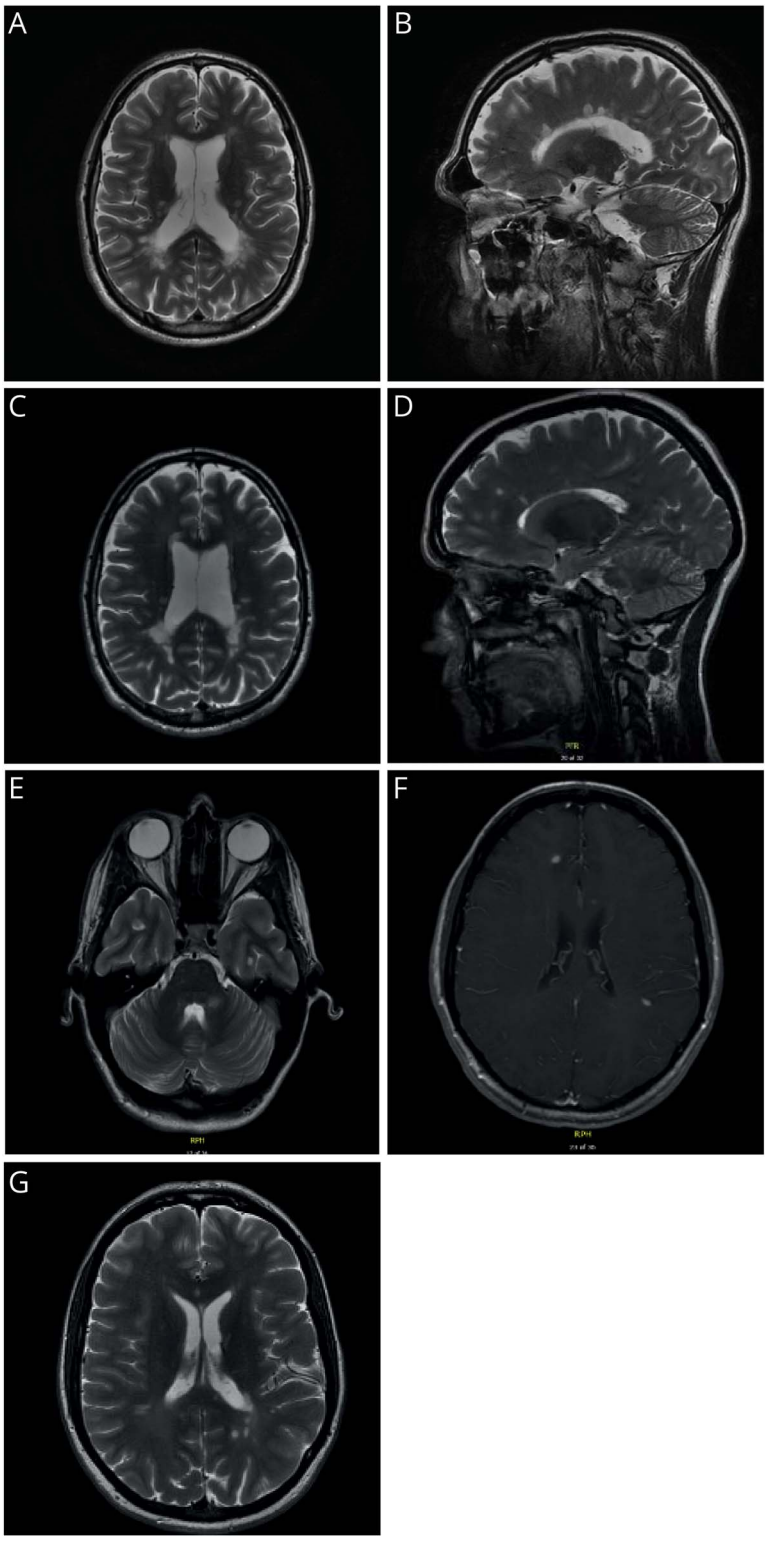
In 2012, he started disease-modifying treatment (DMT) with interferon beta 1-a subcutaneously, but in 2014, because of side effects, he switched to glatiramer acetate. Despite DMT, he experienced relapses, so in 2021, he switched to ocrelizumab and since then he has been relapse free. Owing to treatment with ocrelizumab, he has regular half-yearly checkups. The last MRI scan performed in 2024 had shown numerous typical changes due to demyelination (Figure 2C). His Expanded Disability Status Scale (EDSS)[9] is currently 6.5, primarily because of his ambulation with 2 crutches, but it is difficult to give an objective score because of his simultaneous impairment of the peripheral nervous system.

The propositus' 5th-degree relative, a 34-year-old woman (III.5), was admitted to the emergency department due to headache, sickness, dizziness, and unsteadiness lasting 3 days. She was psychomotor slowed, with horizontal nystagmus and brisk myotatic reflexes except for Achilles, which were diminished; she had mild right-sided hemiparesis and a positive right plantar sign, and strength of feet and toe dorsiflexion was bilaterally limited.

Brain MRI revealed callosal and brainstem demyelinating lesions detected on T2 sequences and gadolinium-enhancing lesions (Figure 2, D–F). VEP showed no abnormalities. CSF analysis revealed the presence of 11 OCBs of IgG and intrathecal IgG synthesis.

She fulfilled the 2010 McDonald criteria for relapsing-remitting MS.[8] The diagnosis was made on clinical grounds and paraclinical laboratory assessments emphasizing the dissemination in space and time (the presence of both gadolinium-enhancing and no enhancing lesions on the baseline MRI). The positive CSF findings also support the inflammatory demyelinating nature of the underlying condition. With EMG, we found prolonged latency of the SNAP

Figure 2 MRI



(A) Brain MRI of the patient (case III.1) with diffuse symmetrical periventricular demyelination lesions (axial T2 sequence). (B) Brain MRI of the patient (case III.1) with multiple callosal demyelination lesions (sagittal T2 sequence). (C) Brain MRI of the patient (case III.1) with diffuse symmetrical periventricular demyelination lesions and dilated lateral ventricles (axial T2 sequence). (D) Brain MRI of the patient (case III.5) revealed callosal demyelinating lesions (sagittal T2 sequence). (E) Brain MRI of the patient (case III.5) revealed demyelinating lesions in the left cerebellar peduncle and in the left paramedian vermis (axial T2 sequence). (F) Brain MRI of the patient (case III.5) revealed gadolinium-enhancing lesions (axial T1 sequence with gadolinium). (G) Brain MRI of the patient (case III.5) revealed new demyelinating lesions on the right side, mainly behind the occipital horn of the lateral ventricle (axial T2 sequence).

(4.7 ms) and reduced sensory nerve conduction velocity (30 m/second) in both median nerves and the left ulnar nerve (45 m/second).MNCVs of the median, ulnar, fibular, and left tibial nerves were within normal limits. We observed prolonged distal latencies of the CMAPs in both median nerves (4.7 and 5.6 ms), the right tibial nerve (7.6 ms), and the right fibular nerve (7.5 ms). The CMAP amplitudes of both fibular and tibial nerves were markedly reduced (0.1 mV). F-wave latencies were prolonged in both ulnar nerves (35 and 38 ms), and F-waves could not be obtained from any of the lower limb

peripheral nerves. Needle EMG of the extensor digitorum brevis revealed firing of only a single MUAP with an amplitude of 0.5 mV. In the tibialis anterior muscle, there was a severely reduced interference pattern with high-amplitude MUAPs (4–8 mV). In the abductor pollicis brevis, spontaneous activity consisting of positive sharp waves was observed.

The DMT therapy with dimethyl fumarate twice daily was initiated, which was later changed to teriflunomide because of the first drug's side effects (nausea, vomiting, and abdominal pain). She has regular clinical and radiologic periodic checkups every year. At the last follow-up in 2024, she was clinically stable, but on MRI, 3 new lesions were seen (Figure 2G). Her clinical status remained stable. Her EDSS 10 is currently 2.0.

We performed a brain MRI on all remaining CMTX-affected family members but the assessment by a neuroradiologist, experienced in detecting demyelinating lesions, did not reveal any evidence of demyelination lesions. We also inspected the exome sequencing data from the 2 patients with MS for the presence of rare pathogenic variants in 245 genes reported to be associated with MS. We have not found any candidate pathogenic variant that could be considered as a genetic risk factor. We have also tested 150 independent patients with MS, 48 familial and 102 sporadic, for pathogenic variants in the GJB1 gene and have not found any pathogenic gene variants. The identified GJB1 variant was submitted to the ClinVar database (with accession ID 373934).

Discussion

More than 200 genetic loci have been suggested to contribute to the MS risk. In the absence of clearly monogenic forms, the polygenic model, in which several genetic variants contribute a modest individual risk to an aggregated risk score, is currently the predominant hypothesis to explain genetic susceptibility. However, to heritability estimated at 50%, it was estimated that 20% is explained by common genetic variants and up to 5% by low-frequency variation in gene coding sequence.[10] Therefore, the identification of new genetic loci could contribute to a better understanding of the genetic architecture of MS.

While MS has increased comorbidity and shared genetic background with other autoimmune diseases,[11] several Mendelian disorders were reported to be associated with increased risk of MS, including familial periodic fever,[12] neurofibromatosis,[13] and CMTX.[3]

In this study, we are focusing on the association between CMTX and MS. Namely, individual case reports and a cohort study of CMTX suggested that carriers of GJB1 pathogenic variants might be at an increased risk of MS. However, as CMTX presents approximately 10% of Charcot-Marie-Tooth disease subtypes, it could be expected to observe the familial occurrence of MS in CMTX families. We report on the first case of familial MS in 2 of 9 members of a CMTX family, which started in the 5th-degree relatives at ages 20 and 34 years, who fulfilled the criteria of RRMS and responded well to DMT. None of the remaining 7 family members carrying the GJB1 pathogenic variant demonstrated any MRI pathologic changes. We have tested the 2 affected members for potential rare pathogenic variants in 245 genes, known to be associated with MS and have not found any evidence for increased genetic risk. On the other hand, we have not found any *GJB1* pathogenic variants in 150 patients with MS, 48 familial and 102 sporadic cases, which implies that *GJB1* pathogenic variants do not represent a common risk factor of MS.

In addition to MS, patients with CMTX1, most frequently male, may experience episodic CNS deficits, including stroke-like episodes manifesting as facial, lingual, or limb weakness; dysarthria or dysphagia; facial or limb numbness; and ataxia at a young age, ranging from infancy to 26 years.[14,15] MRI shows reversible, bilateral, nonenhancing white matter lesions and restricted diffusion most common in bilateral white matter and corpus callosum. The mechanism of CNS manifestation in CMTX can be linked to gap junction channels (GJ). Namely, GJB1 encodes the connexin CX32, which is expressed in noncompact myelin and on the outer membrane of oligodendrocytes, and forms intracellular GJs in large myelin fibers and intercellular GJs with astrocytes.[16] Connexins are crucial in regulating oligodendrocyte differentiation, the microenvironment, energy supply, blood-brain barrier integrity, immune cell infiltration, and the inflammatory response.[17,18]

In conclusion, we provide further support for increased MS risk in the GJB1 pathogenic variant carriers, even if the familial occurrence was coincidental. In addition, we provide evidence that genetic variation in GJB1 is not a common risk factor, neither in patients with familial MS nor in those with sporadic MS.

Author Contributions

M. Menih: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. T. Hojs Fabjan: drafting/revision of the manuscript for content, including medical writing for content; study concept or design. A. Maver: major role in the acquisition of data. B. Peterlin: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

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