Transient, Recurrent, White Matter Lesions in X-linked Charcot-Marie-Tooth Disease With Novel Connexin 32 Mutation

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Background: X-linked hereditary demyelinating neuropathies (Charcot-Marie-Tooth Disease [CMTX]) caused by mutations in the connexin 32 (Cx32) gene account for approximately 10% to 20% of all hereditary demyelinating neuropathies. Mild subclinical central nervous system (CNS) involvement has been previously described, and CMTX patients with transient white matter lesions allied to CNS symptoms have very recently been described. This is of potential interest, as Cx32 is widely expressed in both peripheral nerve and the brain.

Patients: We describe a family with hereditary demyelinating neuropathy and transient CNS symptoms. For this study, family members underwent genotyping and detailed clinical, electrophysiological, and magnetic resonance imaging examination.

Results: We present a CMTX family with a novel mutation in the Cx32 gene. Affected family members show, in addition to the classic polyneuropathy, transient and reversible white matter lesions on magnetic resonance imaging scans, correlating similarly transient CNS symptoms.

Conclusion: Patients with CMTX can present with transient CNS symptoms and marked white matter lesions on magnetic resonance imaging scans.

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EREDITARY demyelinating neuropathies (CMT) are among some of the most frequent hereditary neurological disorders. X-linked hereditary Charcot-Marie-Tooth disease (CMTX) is quite common and accounts for approximately 10% to 20% of all hereditary demyelinating neuropathies. Moreover, most cases have been linked to mutations in the gap junction protein connexin 32 (Cx32). Two connexons, consisting of each of 6 connexins, form a gap junction between 2 cells, and in Schwann cells, Cx32-containing gap junctions make up autoreflexive radial pathways for small molecules. Although Cx32 is also expressed in the liver, pancreas, oligodendrocytes, and astrocytes, no symptoms correlating with liver or pancreatic disease have as yet been observed in CMTX. Subclinical central nervous system (CNS) involvement, detected by, for example, delayed acoustic evoked potential (AEP) and discrete pyramidal signs, has, however, been described and even used to distinguish CMTX from other hereditary demyelinating CMTs. We present a family with CMTX, presenting a novel mutation in the Cx32 gene and showing transient and reversible white matter lesions in magnetic resonance imaging (MRI) concomitantly to variable and reversible CNS symptoms.

PATIENT REPORTS

The family, the pedigree of which is depicted in Figure 1, was recruited from our neuromuscular outpatient clinic.

PATIENTS I-3, I-6, AND III-4

These patients showed no evidence of motor symptoms, foot deformities, or sensory disturbances. Patient II-6 was a newborn girl who died of unknown cause immediately after birth, and another child (III-1) died not long after birth of heart failure.

PATIENT II-3

From age 7 years, this (presently) 43-year-old woman had a slight gait disturbance, discrete foot deformities, and leg cramps. During puberty, she experienced re-
peated attacks of transient paraparesis or monoparesis. In the course of these events, the degree of paresis varied, having an either slowly or rapidly progressive character and persisting for between a few hours and 2 days at the longest. These symptoms recurred at irregular intervals, mainly following trivial respiratory tract infections throughout a period of 10 years. However, she describes no episode in the 10 years preceding our research. Figure 1 presents the clinical and electrophysiological data that were retrieved from the most recent examination. An MRI of the brain at this time showed no abnormalities (data not shown).

**PATIENT III-2**

This young male was born prematurely and had bilateral pes cavus at birth. Milestones were delayed. At age 10 years, a sudden flaccid and fluctuating tetraparesis with predominance in the lower limbs occurred after a football training, and receded within an hour. No evidence of an epileptic seizure, loss of consciousness, or behavior disturbances were documented. He was taking no medication, and neither intoxication nor fasting periods were found in the recent past. A second attack occurred several months later — approximately 3 weeks after a stay in a mountain chalet. At onset, the child displayed behavior abnormalities and disorientation for time and place. Later, severe fluctuating dysarthria, motor aphasia, and a transient spastic hemiparesis developed. Symptoms persisted for 3 hours. Magnetic resonance imaging of the brain showed bilateral white matter lesions in the diffusion-weighted sequence, which were not enhanced by gadolinium in T2-weighted images (Figure 2, A and C). In addition, identical abnormalities were found in both cerebellar peduncles (data not shown). An MRI scan of the whole spinal cord showed no abnormalities. Three months later (Figure 2, B and D), these lesions had almost completely vanished. Table 1 presents results of examination at age 12 years. At age 13 years, the patient died of osteosarcoma.

**PATIENT III-3**

After the premature birth of this boy, his milestones were reached late. From early infancy through puberty, brief and transient episodes of dysarthria, dysphagia, palsy of one or more cranial nerves, as well as rarer attacks leading to gait disturbances, occurred without any discernible cause, sometimes after infections. An MRI scan during one of such episodes showed diffuse hyperintensities in almost all white matter regions, though without gadolinium enhancement (Figure 3A). Abnormalities were most pronounced in parietal white matter and were also documented in the unilateral cerebellar peduncle. Two months later, these MRI abnormalities were virtually completely gone (Figure 3B). One severe episode occurred at age 19 years 5 weeks, following appendectomy after mononucleosis. The young man then suddenly developed bilateral facial palsy, impaired function of the third cranial nerve, as well as severe dysphagia and dysarthria. These symptoms persisted for an hour and reappeared 24 hours later. At this point, MRI scans of brain and spinal cord were performed. The brain MRI scan showed identical abnormalities to those described above, and no abnormalities were observed in the spinal cord. The MRI (6 months after the last episode) shows discrete hyperintensities in the parietal white matter (Figure 3C). To date, clinical examination data are presented in Table 1.

Normal laboratory analysis of all subjects included serum levels of urea, creatinine, creatine kinase, C-reactive protein, homocysteine, serum folate, methylmalonic acid, fasting morning blood glucose, free triiodothyronine, free thyroxine, and thyreotropin; a quantitative estimation of immunoglobulin subclasses G, A, and M serum electrophoresis; anti-*Borrelia burgdorferi* antibody titer, antinuclear antibodies, ultralong fatty acids, and other abnormal laboratory tests.

### Table 1. Clinical and Electrophysiological Data Retrieved From the Most Recent Examinations

<table>
<thead>
<tr>
<th>Case</th>
<th>Foot Deformities</th>
<th>Deafness/Tinnitus</th>
<th>Distal Paresis</th>
<th>Symmetric</th>
<th>Hypertension/Diminished Vibration Sense</th>
<th>Pyramidal Tract Signs</th>
<th>NCV of Tibial Nerve (m/sec)</th>
<th>CMAP of Tibial Nerve (mV)</th>
<th>SNAP of Sural Nerve (µV)</th>
<th>AEP</th>
<th>MEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-3</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>34.6 (&gt;40.6)</td>
<td>6 (&gt;5)</td>
<td>Absent</td>
<td>Abnormal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>III-2</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>33.2 (&gt;45.5)</td>
<td>8.1 (&gt;5)</td>
<td>10 (&gt;6)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>III-3</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>32.5 (&gt;40.6)</td>
<td>5.2 (&gt;5)</td>
<td>6.5 (&gt;6)</td>
<td>Abnormal</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: AEP, acoustic evoked potential; CMAP, compound muscle action potential; MEP, motor-evoked potential; NCV, nerve conduction velocity; ND, not determined; SNAP, sensory nerve action potential.
amino acid distribution, liver enzymes, organic acids, phytic acid, lysosomal enzymes, herpes simplex virus diagnosis, cytomegalovirus diagnosis, varicella zoster virus diagnosis, Epstein-Barr virus diagnosis, measles diagnosis, coxsackievirus diagnosis, human immunodeficiency virus–infection diagnosis, and Treponema pallidum microhemagglutination assay. In patients III-2 and III-3, repeated cerebrospinal fluid analyses were completed. Results were always normal. In patient III-3, fasting cholesterol and triglyceride levels were slightly elevated.

Genomic DNA was extracted from a blood sample of patient III-2 after informed consent had been given. Initially, a PMP22 duplication on chromosome 17p11.2 was excluded with a set of polymorphic microsatellite markers, and absence of a specific junction fragment was ascertained by using polymerase chain reaction (PCR)–based techniques. As the family pedigree was compatible with an X-linked–type transmission, molecular diagnosis was accomplished by direct sequencing of the coding region of the Cx32 gene (ABI PRISM 373A Automated Fluorescent DNA Sequencer; Applied Biosystems, Weiterstadt, Germany) and revealed a deletion of a glutamic acid in codon 102 (304_306delGAG); this mutation had not been reported previously. The deletion was shown to segregate with the phenotype in this family with the patient’s mother (II-1) being a heterozygous carrier. DNA samples from other family members or members of the nucleus family and 100 apparently unrelated normal controls were also tested under appropriate single-stranded conformational polymorphism conditions and permitted to exclude a possible polymorphism.

Figure 2. Magnetic resonance images of patient III-2, with diffusion-weighted (A and B) and T2-weighted (C and D) transverse sections. C, Abnormal hyperintensities in parietal white matter were documented. D, Three months later, a virtually complete disappearance of findings was observed.
We present a family with a CMTX-type polyneuropathy also presenting transient CNS symptoms, carrying a novel mutation in the \textit{Cx32} gene. Clinical and electrophysiological examinations showed the typical picture of a slowly progressive demyelinating neuropathy related to \textit{Cx32} mutations. Remarkably, transient non–gadolinium-enhanced white matter abnormalities, correlating with transient clinical neurological deficits, were observed in this family. Symmetry and sparing of U fibers on MRI are compatible with inherited white matter disease. More interestingly, clinical signs included paresis of one or more cranial nerves, indicating brainstem pathology. After 48 hours at most, clinical pathology had regressed in all cases. Follow-up MRI scans showed that the abnormalities observed in T2-weighted sequences were markedly reduced. Irrespective of the transient abnormalities and consistent with small permanent residual MRI findings (Figure 3C), clinical and electrophysiological examination showed mild persistent signs of CNS involvement in our patients.

Cosegregation of 2 different genetic defects, one causing CMTX, the other a white matter disease of the CNS, seems unlikely, as to date, 3 independent reports, including ours, describe the association of CNS symptoms and concomitant transient white matter lesions with CMTX (summarized in Table 2). Furthermore, many reports document subclinical involvement of CNS in CMTX (eg, delayed AEP, motor evoked potential, and somatosensory evoked potential). Also, as \textit{Cx32} is expressed in oligodendrocytes, it is conceivable that certain \textit{Cx32} mutations might lead to white matter abnormalities and CNS symptoms.

Different binding partners and different cellular localization in the CNS and peripheral nervous system (PNS) may be the reason why usually the PNS is predominantly affected in CMTX. Certain \textit{Cx32} mutations, like the one described here, may however cause obvious CNS disease. As \textit{Cx32} knockout mice have a normal CNS, these \textit{Cx32} mutations leading to CNS symptoms may act transdominant negative on other connexins known to be expressed in oligodendrocytes such as \textit{Cx47} and \textit{Cx27}. The existence of such transdominant negative effects has been described in vitro.\textsuperscript{11,12}

There is as yet no hotspot for \textit{Cx32} mutations associated with CNS symptoms. The described mutations which have been associated with CNS disease are found to correspond to amino acids of the extracellular as well as intracellular or transmembrane regions of the protein. Thus the dominant negative effect is probably either heteromeric or heterotopic.

In analogy to channelopathies, altered gating properties of connexons with mutated \textit{Cx32} could theoretically give rise to transient CNS symptoms. Situations of metabolic stress may further alter the function of gap junctions and cause transient symptoms.

Whatever the exact pathomechanism turns out to be, it is important to remember that there are clinical examples, as well as a good theoretical background supporting the idea that the second most frequent hereditary de-
myelinating neuropathy can not only lead to subclinical CNS involvement, but can cause patent CNS disease.

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REFERENCES