Selective Bilateral Vestibular Neuropathy in a Turkish CMT1B Family With a Novel MPZ Mutation

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Abstract

Purpose of Review
To report the findings in 12 members over 3 generations of a family with dominantly inherited Charcot-Marie-Tooth disease (CMT1B) due to a novel MPZ mutation, who all had moderately severe selective impairment of vestibular function with normal hearing. Methods used were video head impulse testing of the function of all 6 semicircular canals, Romberg test on foam, nerve conduction studies, and whole exome and Sanger sequencing.

Recent Findings
All affected patients had a demyelinating neuropathy and a novel MPZ mutation: c.362A>G (chr1: 161276584, p.D121G). All also had normal hearing for age but a moderately severe impairment of semicircular canal function and a positive Romberg test on foam.

Summary
Some CMT mutations can impair vestibular function, presumably because of a vestibular nerve involvement but spare hearing. In such patients, impairment of vestibular function and impairment of proprioception contribute to imbalance.

Although some patients with Charcot-Marie-Tooth disease (CMT) have obvious hearing impairment from auditory nerve involvement, vestibular impairment will not be obvious because the patient already has imbalance from distal weakness and somatosensory impairment.1,2 Some patients with CMT—CMT1B/MPZ—3 have only auditory impairment, whereas others—CMT4C/H3TC2–5 or CMT4D/NDRG1–6—have severe, early impairment of both auditory and vestibular function. Here, we report 12 members in 3 generations of a Turkish family with CMT1B demyelinating neuropathy due to a novel MPZ mutation, who have selective bilateral vestibular impairment with normal hearing. In some, vestibular rather than proprioceptive impairment seems the principal cause of postural instability.
Methods

Genetic Testing
Sample Collection and DNA Extraction
Minimum 3 mL of peripheral blood was collected in ethylenediaminetetraacetic acid. The DNA extraction was performed by the commercial QIAcube method (Qiagen, Hilden, Germany) according to the recommended extraction and purification protocols. The DNA was stored −20 °C.

Fragment Analysis for HNPP by STR Markers
The index case (IV.3) was tested for 17p11.2–12 duplication by fragment analysis.

Multiplex Ligation-Dependent Probe Amplification
The multiplex ligation-dependent probe amplification (MLPA) assay was used for 4 family members (III.6, IV.4, IV.3, and V.2). The DNA samples were investigated by SALSA MLPA Pro-bmix P033-B4 CMT1 (MRC-Holland, Holland), which contains 38 probes specific for PMP22, KIF1b genes, and control regions according to the producer’s recommendations.

Whole Exome Sequencing
The DNA of index case was studied by whole exome sequencing (WES), and a novel, heterozygous variation of MPZ gene (ENST00000533357.4) was found: c.362A>G (chr1: 161276584, p.N121G), confirmed by Sanger sequencing. The variation is classified as likely pathogenic according to the ACMG criteria.

Sanger Sequencing
Conventional Sanger sequencing was used for the confirmation of MPZ c.362A>G variation and for screening family members (III.6, IV.3, IV.4, IV.8, and V.2).

Vestibular Function
Function of each semicircular canal (SCC) was tested in turn by vestibular testing (III.6, IV.3, IV.4, IV.8, IV.9, and V.5). VOR gain in response to rapid, passive, head accelerations in the plane of each SCC—the Head Impulse Test (GN Otometrics, Tastrup, Denmark)—indicated that both somatosensory and vestibular impairment. Of the 9 patients, 5 (III.6, IV.3, IV.5, IV.9, and IV.10) had positive foam Romberg test, indicating proprioceptive impairment. On a foam surface, 5 (IV.3, IV.8, IV.9, IV.10, and V.5) could stand with eyes open but not with eyes closed—i.e., these 5 had a positive firm Romberg test.

Balance
Of 12 patients, 9 attempted Romberg testing. On a firm surface, all 9 could stand with eyes open, but 5 (III.6, IV.3, IV.5, IV.9, and IV.10) could not do so with eyes closed—i.e., these 5 had a positive firm Romberg test.

Family Tree and Genetics
The index case (IV.3) has a consanguineous marriage with a fourth degree relative, the only consanguineous marriage in the family (figure 2). There is no other genetic disorder in the family. The results of specific fragment analysis targeting for HNPP-related 17p11.2–12 deletion and MLPA analysis for PMP22 are all normal. The WES analysis identified MPZ c.362A>G (chr1: 161276584, p.D121G) variation. Sanger sequencing confirmed the presence of heterozygous MPZ c.362A>G variation in the index case (IV.3) and was used for screening of other family members. The variation was also found in the index case’s mother (III.6), son (V.1), brothers (IV.5 and IV.10), sisters (IV.8 and IV.9), and nephews (V.5, V.6, V.7). They all have polyneuropathy. Co-segregation of the variation and the disorder is revealed in the family. Therefore, the variation is considered as likely pathogenic according to the ACMG criteria: PS4, PM1, PM2, and PP3. This result was confirmed in the index case (IV.3) at an independent laboratory.

Results
Clinical Features
The initial problem was imbalance, starting in infancy in 7. All except 3 (V.1, V.12, and V.13) had pes cavus; 4 with pes cavus (IV.5, V.5, V.6, and V.7) also had hammer toes. None had appreciable weakness. All those old enough for reliable sensory testing had distal impairment of vibration sense in the lower limbs. None had lower limb tendon reflexes. None had asymptomatic hearing loss; 3 (III.6, IV.3, and IV.10) had audiograms—all 3 normal for age.

Nerve Conduction Studies
Of the 12 mutation-positive patients tested (table), 11 had marked slowing of motor conduction (10–26 m/s) and also of sensory conduction (20–28 m/s) in the 5 patients (IV.3, IV.8, IV.9, V.1, and V.12) with sensory action potentials.

Vestibular Function
Every patient had some impairment of SCC function in one or more canals. There appeared to be no association of extent or severity with patient age (table). The oldest patient (III.6) had impairment of all 6 SCCs (figure 1), whereas the youngest (V.7) who could have 6 SCC testing had impairment of 4 SCCs. In all patients, the SCC impairment was bilateral and of moderate severity with gains of 0.57–0.85 for the lateral canals (normal >0.85), 0.46–0.80 (normal >0.80) for the anterior canals, and 0.30–0.70 for the posterior canals (normal >0.70).

Balance
Of the 12 patients, 9 attempted Romberg testing. On a firm surface, all 9 could stand with eyes open, but 5 (III.6, IV.3, IV.5, IV.9, and IV.10) could not do so with eyes closed—i.e., these 5 had a positive firm Romberg test, indicating proprioceptive impairment. On a foam surface, 5 (IV.3, IV.8, IV.9, IV.10, and V.5) could stand with eyes open but not with eyes closed—i.e., these 5 had a positive foam Romberg test, indicating vestibular impairment. Of the 9 patients, 5 (III.6, IV.3, IV.5, IV.9, and IV.10) had positive firm and foam Romberg tests, indicating that both somatosensory and vestibular impairment contributed to their imbalance.
| Generation degree (gender, age) | Nerve conduction studies | VOR gain | Romberg test score |
|---------------------------------|--------------------------|----------|-------------------|
|                                 |                          | Lateral canal; normal >0.85 | Anterior canal; normal >0.80 | Posterior canal; normal >0.70 | Firm | Foam |
|                                 | Ulnar motor | Ulnar sensory |                         |                             | Left | Right | Left | Right | Left | Right | EO | EC | EO | EC |
| Affected                        |             |              |                          |                             |      |       |      |       |      |       |    |    |    |    |
| III.6 (F, 55)                  | 5.4 | 4 | 21.6 | 0 | 0 | 0 | 0.75 s | 0.67 s | 0.56 s | 0.46 s | 0.30 s | 0.58 s | 3 | 4 | 4 | 4 |
| IV.3 (F, 27)                   | 6.5 | 4.0 | 22.3 | 10 | 4.1 | 28 | 0.57 s | 0.57 s | 0.74 s | 0.71 s | 0.48 s | 0.46 s | 2 | 4 | 3 | 4 |
| IV.5 (M, 32)                   | 6.7 | 4.1 | 20 | 0 | 0 | 0 | 0.85 s | 0.90 s | 0.86 s | 0.80 s | 0.55 s | 0.49 s | 2 | 4 | 4 | 4 |
| IV.8 (F, 22)                   | 8.3 | 3.9 | 18.2 | 6.5 | 4.7 | 23.4 | 0.79 s | 0.69 s | 1.02 s | 1.26 s | 0.82 s | 0.76 s | 1 | 3 | 2 | 4 |
| IV.9 (F, 19)                   | 3.8 | 3.9 | 25.9 | 16.2 | 4.2 | 25.3 | 0.70 s | 0.76 s | 0.64 s | 0.79 s | 0.56 s | 0.62 s | 1 | 4 | 2 | 4 |
| IV.10 (M, 17)                  | 4.7 | 5.5 | 21 | 0 | 0 | 0 | 0.87 s | 0.96 s | 0.51 s | 0.92 s | 0.62 s | 0.62 s | 1 | 4 | 2 | 4 |
| V.1 (M, 5)                     | 5.9 | 4.2 | 10.1 | 4.9 | 3.8 | 19.7 | 0.81 | 0.81 | 0.72 s | 0.71 s | 0.85 s | 1.07 | 0.66 s | 0.62 s | 1 | 1 | 2 | 4 |
| V.5 (F, 14)                    | 6.7 | 4.7 | 22 | 0 | 0 | 0 | 0.86 s | 0.89 s | 0.76 s | 0.67 s | 0.65 s | 0.70 s | 1 | 1 | 4 | 4 |
| V.6 (M, 12)                    | 3.3 | 3.5 | 20 | 0 | 0 | 0 | 0.66 s | 0.85 s | 0.80 s | 0.76 s | 0.62 s | 0.66 s | 1 | 1 | 4 | 4 |
| V.7 (M, 10)                    | 5.1 | 3.5 | 21 | 0 | 0 | 0 | 0.78 | 0.86 | 1.05 | 1.26 | 0.67 | 1.00 | 0.84 | 0.60 | 1 | 1 | 1 | 1 |
| V.12 (M, 5)                    | 3.6 | 3.1 | 21.2 | 8.2 | 2.9 | 24.1 | 0.83 | 0.88 | 1.05 | 1.26 | 0.67 | 1.00 | 0.84 | 0.60 | 1 | 1 | 1 | 1 |

Unaffected

| IV.4 (F, 40) | 1.05 | 1.26 | 0.67 | 1.00 | 0.84 | 0.60 | 1 | 1 | 1 | 1 |
| V.2 (F, 3)   | 0.83 | 0.88 | 1.05 | 1.26 | 0.67 | 1.00 | 0.84 | 0.60 | 1 | 1 | 1 | 1 |

Abbreviations: EC = eyes closed; EO = eyes open; SCC = semicircular canal; VOR = vestibulo-ocular reflex.
Motor and sensory conduction velocities are all in the demyelinating range. With EC, none of the patients could stand on foam. The deficits in VOR gain are shown in bold. Catch-up saccades (s) can be a confirmatory sign of VOR deficit but are not always present, even when the VOR is defective. In 2 patients (III.6 and IV.9), VOR from all 6 canals were defective. In the others, there was no obvious pattern as to which canals were involved and which were not. Patient V.1 (age 5) could cooperate only with lateral SCC canal testing.
Each clinically affected patient is also electrophysiologically affected (indicated by dotted symbols) and is MPZ mutation positive (indicated by crimson shading and a plus sign “+”). Conversely, each MPZ mutation-positive patient is clinically and electrophysiologically affected. Patient IV.4 (unaffected) has 5 unaffected children; only the youngest (age 4) is indicated with a symbol; the ages of the other 4 are appended. The only exception is patient V.13, a clinically affected, MPZ-positive 3 years old, too young to cooperate with electrophysiologic or vestibular testing. The arrow indicates index case.

A typical example, from patient III.6, a woman aged 55. Vestibulo-ocular reflex (VOR) gain is reduced from each of the 6 semicircular canals (SCCs). Head velocity is shown in blue for 30 leftward head impulses and in orange for 30 rightward head impulses in the plane of each SCC. VOR eye velocity is shown in green. Catch-up saccades compensating for any VOR inadequacy are shown in red. This patient happens to make fewer catch-up saccades in response to head impulses in the right anterior-left posterior canal plane. In the centre of the figure, VOR gains are illustrated as a polar plot.
Discussion

CMT1B is a hereditary demyelinating neuropathy due to one of more than 200 possible mutations in the MPZ gene. In patients with early onset disease, the neuropathy is demyelinating.\(^1\) In this family with a novel MPZ mutation (exon 3, c.362A>G; p.D121G), there was a contrast between the variable vestibular impairment and the normal hearing. All our patients had bilateral impairment of SCC function affecting some but not all SCCs. There was no relationship between the patient age and vestibular impairment severity, suggesting that the vestibular impairment is static.

Poretti et al.\(^1\) reported bilateral vestibular impairment affecting both otolith function (tested with cervical vestibular-evoked myogenic potentials) and lateral SCC function (tested with head impulses using the scleral search coil method) in 15 patients with CMT. Of these 15, 13 had a demyelinating neuropathy, 4 had CMT1A (PMP22 dupl), 3 had CMTX1 (GJB1x32), one each had DI-CMT (MPZ), CMT2 (undef), CMT4D (undef), and 5 had clinically and genetically undefined CMT but a positive family history. In general, video Head Impulse Test gains were reduced and cervical vestibular evoked myogenic potential latency was increased; findings suggesting that these patients had a demyelinating vestibular and peripheral neuropathy. Of these 15 patients, 6 had auditory and vestibular impairment. In Dejerine-Sottas syndrome (motor conduction velocity 3–4 m/s) because of a T420C mutation predicting an L71P variant in exon 2 of the MPZ gene (c.193A>G; p.Thr65Ala), both auditory and vestibular function were impaired.\(^1\) In the other with a missense mutation in exon 3 of the MPZ gene (c.434A>G; p.Tyr145Ser), neither auditory or vestibular function were impaired.\(^1\)

Although we cannot distinguish vestibular end-organ from vestibular nerve involvement causing vestibular impairment, physiologic and histologic data indicate that vestibular nerve demyelination is the cause. The marked delay in cVEMP suggests demyelination.\(^1\) Otopathologic examination of 2 separate CMT1A/MPZ cases showed extensive demyelination of the vestibular and cochlear nerves with preservation of receptor hair cells. In one case with a heterozygous missense variant in exon 2 of the MPZ gene (c.193A>G; p.Thr65Ala), both auditory and vestibular function were impaired.\(^1\) In the other with a missense mutation in exon 3 of the MPZ gene (c.434A>G; p.Tyr145Ser), neither auditory or vestibular function was evaluated.\(^1\)

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Disclosure

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