Selective bilateral vestibular neuropathy in a Turkish CMT1B family with a novel MPZ mutation

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ABSTRACT

**Purpose of review.** To report 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, whole exome and Sanger sequencing.

**Recent finding.** 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 due a vestibular nerve involvement but spare hearing. In such patients impairment of vestibular function as well as impairment of proprioception contributes to imbalance.

INTRODUCTION

While some CMT patients have obvious hearing impairment from auditory nerve involvement, vestibular impairment will not be obvious, since the patient already has imbalance from distal weakness and somatosensory impairment.¹ Some CMT patients - CMT1B/MPZ,² have only auditory impairment whereas others - CMT4C/H3TC2,³⁴ or CMT4D/NDRG1⁵ 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.
MATERIALS AND METHODS

Genetic testing
Sample Collection and DNA Extraction. Minimum 3 ml of peripheral blood was collected in EDTA. The DNA extraction is done by the commercial QIAcube method (Qiagen, 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 (MLPA). The 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 probemix 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 (WES). 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 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, IV.9, V.1, V.2 and V.5).

Vestibular testing
Function of each semicircular canal (SCC) was tested in turn by measuring the vestibulo-ocular reflex (VOR) gain in response to rapid, passive, head accelerations in the plane of each SCC – the Head Impulse Test (GN Otometrics, Tastrup, Denmark). The test assesses both VOR gain and any catch-up saccades compensating for low VOR gain.

Balance Testing
In the modified Romberg test the patient first stands on a firm surface (the floor), then on a foam surface, with eyes open, then closed. Postural stability is observed
for 30 seconds. Sway is graded: 1 = minimal, 2 = mild, 3 = moderate 4 = potential fall.8
The study was approved by the Hospital’s institutional review board. Each adult
patient and the parent of each child gave written permission for testing and for
publication of the results.

RESULTS

Clinical features
The initial problem was imbalance, starting in infancy in 7.9 All except 3 (V.1, V.12,
and V.13) had pes cavus; 4 with pes cavus (IV.5, V.5, V.6, 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 symptomatic 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 1) 11 had marked slowing of motor
conduction (10-26m/s) and also of sensory conduction (20-28m/s) in the 5 patients
(IV.3, IV.8, IV.9, V.1, 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 1). The
oldest patient (III.6) had impairment of all 6 SCCs (Fig 1) while 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 0.66 to 0.81 for the
lateral canals (normal > 0.85), 0.47 to 0.79 (normal > 0.80) for the anterior canals
and 0.30 to 0.76 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, 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, 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, IV.10) had positive firm and foam Romberg tests, indicating that both somatosensory and vestibular impairment contributed to their imbalance.\textsuperscript{8}

**Family tree and genetics**

The index case (IV.3) has a consanguineous marriage with a 4\textsuperscript{th} degree relative, the only consanguineous marriage in the family (Fig 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 index case (IV.3) and was used for screening of other family members. The variation was also found in index case’s mother (III.6), son (V.1), brothers (IV.5, IV.10), sisters (IV.8, 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. So the variation is considered as likely pathogenic according to ACMG criteria: PS4, PM1, PM2, PP3.\textsuperscript{6} This result was confirmed in the index case (IV.3) at an independent laboratory.

**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.\textsuperscript{10} In this family with a novel MPZ mutation (exon 3, c.362A>G; p.D121G) there was a contrast between the invariable 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 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 CMT patients.\textsuperscript{1} 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 (vHIT) gains were reduced and cervical vestibular evoked myogenic potential latency was increased findings suggesting that these patients had a demyelinating vestibular as well as peripheral neuropathy. Of these 15 patients, 6 had auditory as well as vestibular impairment. In Dejerine-Sottas syndrome (motor conduction velocity 3-4 m/s) due to a T420C mutation predicting an L71P amino acid change of PMP22 protein there can be total bilateral absence of lateral SCC canal function and a 40-50 dB sensorineural hearing loss.\(^{11}\)

While 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 cVEMPs 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.\(^{12}\) In the other with a missense mutation in exon 3 of the MPZ gene (c.434A>C; p.Tyr145Ser) neither auditory or vestibular function was evaluated.\(^{13}\)

**TAKE HOME POINTS**

- Clinicians should consider a vestibular contribution to CMT patients’ imbalance
- Video Head Impulse Testing can accurately measure function of each of the 6 semicircular canals
- It is well tolerated and takes only about 20 minutes

Appendix 1: Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
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<tbody>
<tr>
<td>Gülden Akdal</td>
<td>Dokuz Eylül University, İzmir, Turkey</td>
<td>Design and conceptualization of study, drafting intellectual content of manuscript</td>
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<tr>
<td>Name</td>
<td>Institution</td>
<td>Contributions</td>
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<tr>
<td>Koray Koçoğlu</td>
<td>Dokuz Eylül University, İzmir, Turkey</td>
<td>Data collection, analysis and presentation</td>
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<tr>
<td>Elçin Bora</td>
<td>Dokuz Eylül University, İzmir, Turkey</td>
<td>Genetic testing and analysis</td>
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<td>Altuğ Koç</td>
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<td>Gábor Michael Halmágyi</td>
<td>Royal Prince Alfred Hospital, Sydney, Australia</td>
<td>Design and conceptualization of study, drafting intellectual content of manuscript</td>
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FIGURE LEGENDS

Table 1. Nerve Conduction, Video Head Impulse and Romberg Test. Results for 11/12 affected patients. Motor and sensory conduction velocities are all in the demyelinating range. With eyes closed none of the patients could stand on foam. The deficits in vestibulo-ocular reflex (VOR) gain are shown in bold. Catch-up saccades (s) are 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.
Figure 1. Family Tree. Each clinically affected patient is also electro-physiologically 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 electro-physiologically 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 year old, too young to cooperate with electrophysiological or vestibular testing. The arrow indicates index case.
Figure 2. Video Head Impulse Testing. A typical example, from patient III.6, a female aged 55. VOR gain is reduced from each of the 6 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.
REFERENCES


Table 1. Nerve conduction, head impulse and Romberg test results

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<tr>
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<td>Amplitude (mV)</td>
<td>Latency (ms)</td>
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s = catch-up saccade compensating for vestibulo-ocular reflex deficit. EO = Eyes Open; EC = Eyes Closed.
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