OPEN

Neurology®
Clinical Practice

AMERICAN ACADEMY OF NEUROLOGY®

A peer-reviewed clinical neurology journal for the practicing neurologist
An Official Journal of the American Academy of Neurology

Neurology: Clinical Practice Publish Ahead of Print
DOI: 10.1212/CPJ.0000000000000930

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

Gülden Akdal MD, Koray Koçoğlu MSc, Elçin Bora MD PhD, Altuğ Koç MD PhD, Ayfer Ülgenalp PhD, Mithat Bedir MD, Rahmi Tümay Ala MD, Esra Battaloğlu PhD, Günay Kırkım PhD, İhsan Şükrü Şengün MD, Gábor Michael Halmágyi MD

The Article Processing Charge was funded by the authors.

Gülden Akdal, Dokuz Eylül University, Department of Neurology and Department of Neuroscience, Institute of Health Sciences, İzmir, Turkey
Koray Koçoğlu, Dokuz Eylül University, Department of Neuroscience, Institute of Health Sciences İzmir, Turkey
Elçin Bora, Dokuz Eylül University, Department of Medical Genetics, İzmir, Turkey
Altuğ Koç, Dokuz Eylül University, Department of Medical Genetics, İzmir, Turkey
Ayfer Ülgenalp, Dokuz Eylül University, Department of Medical Genetics, İzmir, Turkey
Mithat Bedir, Mardin State Hospital, Department of Neurology, Mardin, Turkey
Rahmi Tümay Ala, Dokuz Eylül University, Department of Neurology, İzmir, Turkey
Esra Battaloğlu, Boğaziçi University, Department of Molecular Biology and Genetics, İstanbul, Turkey
Günay Kırkım, Dokuz Eylül University, Department of Otorhinolaryngology, Hearing, Speech and Balance Unit, İzmir, Turkey
İhsan Şükrü Şengün, Dokuz Eylül University, Department of Neurology, İzmir, Turkey
Gábor Michael Halmágyi, University of Sydney, Royal Prince Alfred Hospital, Department of Neurology, Sydney, Australia

Search Terms: Charcot-Marie-Tooth, Hereditary Polyneuropathy, Vestibular.
Publication history: Nil
Submission Type: **Article**
Title Character count: **93 (with spaces)**
Number of Tables: **1**
Number of Figures: **2**
Word count of Abstract: **143**
Word Count of Paper: **1,232**
Corresponding author: GM Halmágyi, gmh@icn.usyd.edu.au

Disclosures:
Gülden Akdal - reports no disclosures,
Koray Koçoğlu - reports no disclosures,
Elçin Bora - reports no disclosures,
Altuğ Koç - reports no disclosures,

Ayfer Ülgenalp - reports no disclosures,
Mithat Bedir - reports no disclosures,
Rahmi Tümay Ala - reports no disclosures,
Esra Battaloğlu - reports no disclosures,
Günay Kırkım, reports no disclosures,
İhsan Şükrü Şengün - reports no disclosures,
Gábor Michael Halmágyi - was formerly unpaid consultant to GN Otometrics for development of video head impulse testing.

Statistical Analysis conducted by Dokuz Eylül University scientists Gülden Akdal, MD and Koray Koçoğlu, MSc.

Study funding by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under Grant Number (216S658). The content is merely the responsibility of the authors and does not necessarily represent the views of TÜBİTAK.

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,[3,4] or CMT4D/NDRG1[5] 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^{o}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.[6]

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).[7] 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 contibuted to their imbalance.[8]

Family tree and genetics

The index case (IV.3) has a consanguineous marriage with a 4th 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.[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.[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.[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

Name	Location	Contribution
Gülden Akdal	Dokuz Eylül University, İzmir, Turkey	Design and conceptualization of study, drafting intellectual content of manuscript

Koray Koçoğlu	Dokuz Eylül University, İzmir, Turkey	Data collection, analysis and presentation
Elçin Bora	Dokuz Eylül University, İzmir, Turkey	Genetic testing and analysis
Altuğ Koç	Dokuz Eylül University, İzmir, Turkey	Genetic testing and analysis
Ayfer Ülgenalp	Dokuz Eylül University, İzmir, Turkey	Genetic testing and analysis
Mithat Bedir	Mardin State Hospital, Mardin, Turkey	Clinical and neurophysiological evaluation
Rahmi Tümay Ala	Dokuz Eylül University, İzmir, Turkey	Clinical and neurophysiological evaluation
Esra Battaloğlu	Boğaziçi University, İstanbul, Turkey	Genetic testing and analysis
Günay Kırkım	Dokuz Eylül University, İzmir, Turkey	Audiological evaluation
İhsan Şükrü Şengün	Dokuz Eylül University, İzmir, Turkey	Clinical and neurophysiological evaluation
Gábor Michael Halmágyi	Royal Prince Alfred Hospital, Sydney, Australia	Design and conceptualization of study, drafting intellectual content of manuscript

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.

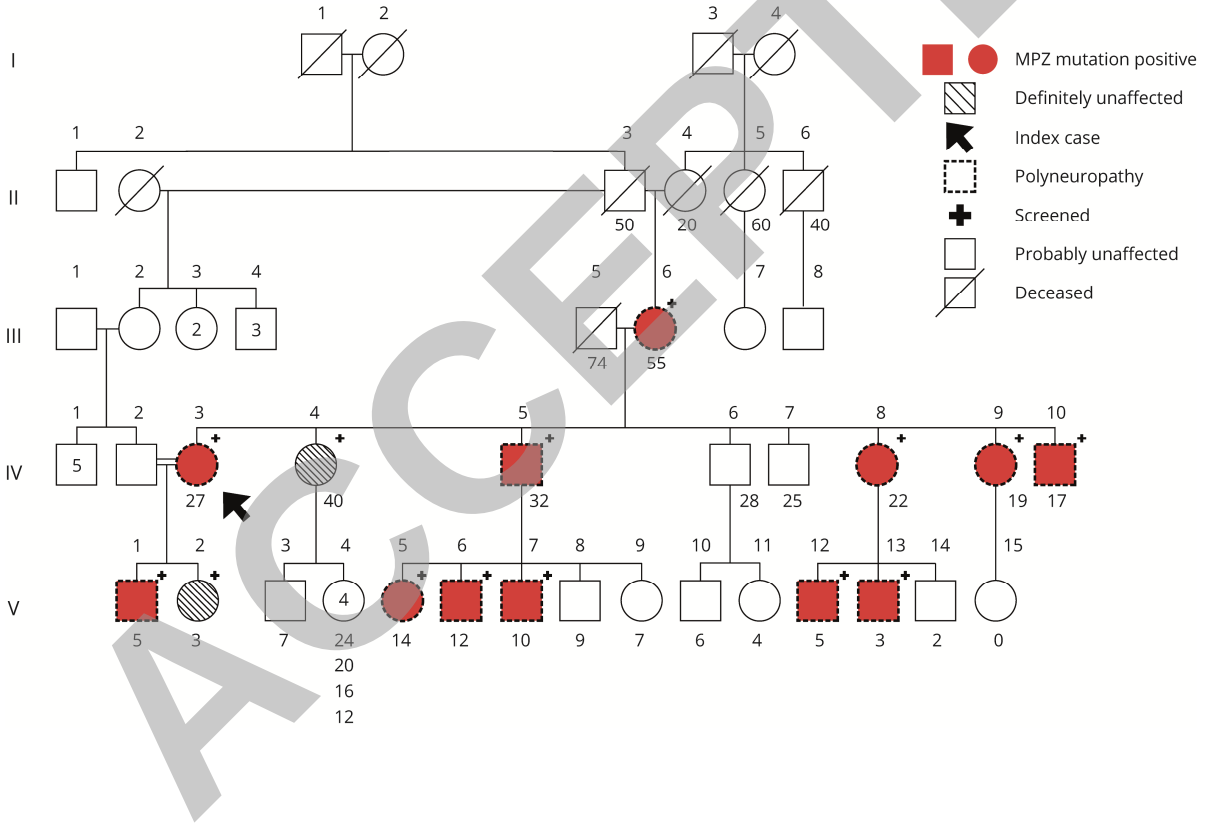
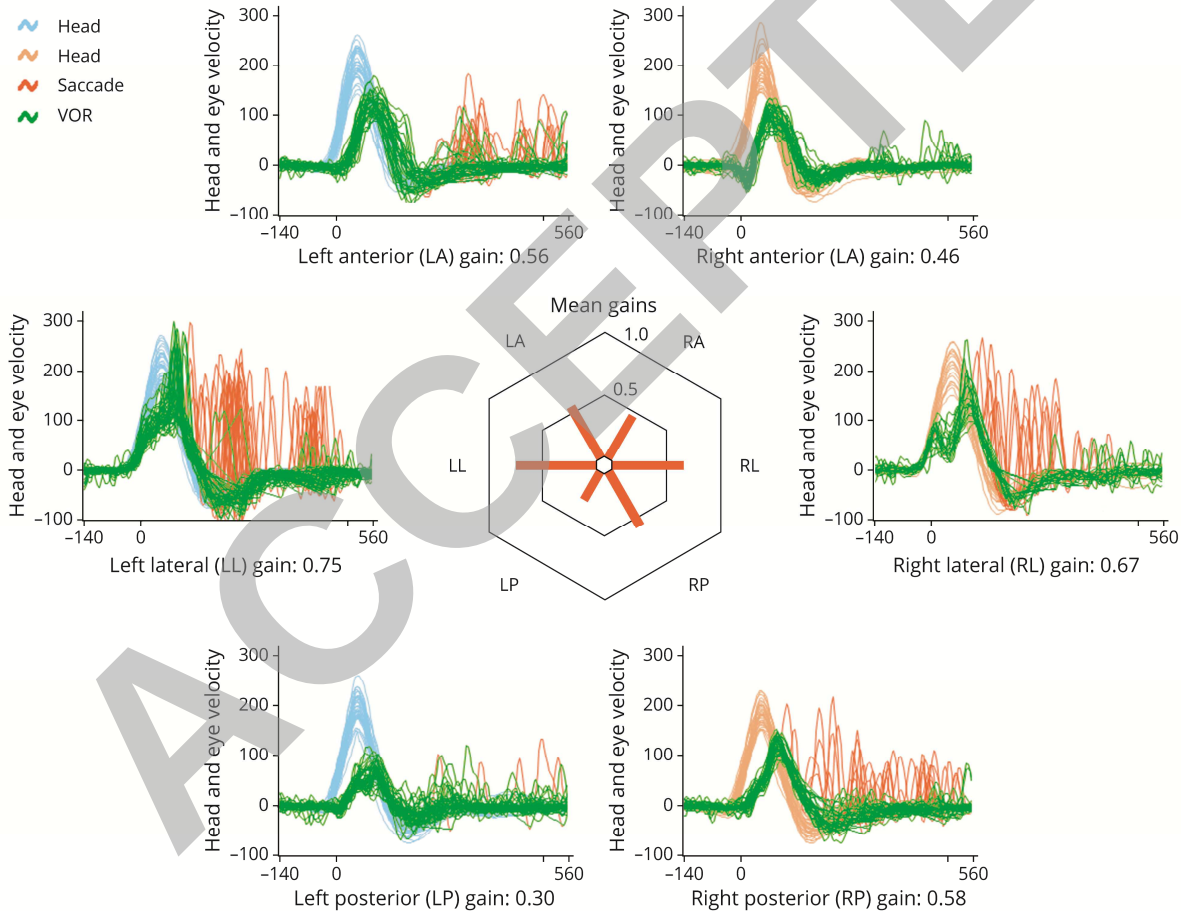


Figure 2. Video Head Impulse Testing. A typical example, from patient III.6, a female aged 55. VOR gain is reduced from each of the 6 SCCs. Head velocity is shown in blue for 30 leftward head impulses and in orange for 30 rightward head impulses in the plane of each SCC. VOR eye velocity is shown in 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

1. Poretti A, Palla A, Tarnutzer AA, et al. Vestibular impairment in patients with Charcot-Marie-tooth disease. Neurology 2013;80:2099–2105.
2. Duan X, Gu W, Hao Y, et al. A Novel Asp121Asn mutation of myelin protein zero is associated with late-onset axonal Charcot–Marie–Tooth disease, hearing loss and pupil abnormalities. Front Aging Neurosci Epub 2016 Sep 22.
3. Pérez-Garrigues H, Sivera R, Vílchez JJ, Espinós C, Palau F, Sevilla T. Vestibular impairment in Charcot–Marie–Tooth disease type 4C. J Neurol Neurosurg Psychiatry 2014;85:824–827.
4. Sivera R, Cavalle L, Vílchez JJ, Espinós C, Pérez Garrigues H, Sevilla T. Audiological findings in Charcot-Marie-Tooth disease Type 4C. J Int Adv Otol 2017;13:93–99.
5. Dačković J, Keckarević-Marković M, Komazec Z, et al. Hereditary motor and sensory neuropathy Lom type in a Serbian family. Acta Myol 2008;27:59–62.
6. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–423.
7. Halmagyi GM, Curthoys IS. The video head impulse test in clinical practice. Neurol Sci Neurophysiol 2018;35:1–5.
8. Horak FB. Clinical measurements of postural control in adults. Phys Ther 1987;67:1881–1885.
9. Estilow T, Glanzman AM, Burns J, et al. Balance impairment in pediatric Charcot-Marie-Tooth disease. Muscle Nerve 2019;60:219–221.
10. Callegari I, Gemelli C, Geroldi A, et al. Mutation update for myelin protein zero-related neuropathies and the increasing role of variants causing a late-onset phenotype. J Neurol 2019;266:2629–2645.
11. Jen J, Baloh RH, Ishiyama A, Baloh RW. Dejerine-Sottas syndrome and vestibular loss due to a point mutation in the *PMP22* gene. J Neurol Sci 2005;237:21–24.

12. Nadol JB Jr, Hedley-Whyte ET, Amr SS, O Apos Malley JT, Kamakura T. Histopathology of the inner ear in Charcot-Marie-Tooth syndrome caused by a missense variant (p.Thr65Ala) in the *MPZ* gene. Audiol Neurootol 2018;23:326-334.
13. Starr A, Michalewski HJ, Zeng FG, et al. Pathology and physiology of auditory neuropathy with a novel mutation in the *MPZ* gene (Tyr145->Ser). Brain 2003;126:1604–1619.

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

Generation Degree (gender, age)	Nerve conduction studies						Vestibulo-ocular reflex gain						Romberg test score			
	Ulnar motor			Ulnar sensory			Lateral canal *normal > 0.85*		Anterior canal *normal > 0.80*		Posterior canal *normal > 0.70*		Firm		Foam	
	Amplitude (mV)	Latency (ms)	Velocity (m/s)	Amplitude (μV)	Latency (ms)	Velocity (m/s)	Left	Right	Left	Right	Left	Right	EO	EC	EO	EC
Affected																
III.6 (F55)	5.4	4	21.6	0	0	0	**0.75 s**	**0.70 s**	**0.56 s**	**0.51**	**0.30**	**0.58 s**	3	4	4	4
IV.3 (F27)	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 (M32)	6.7	4.1	20	0	0	0	0.85 **s**	0.90 **s**	0.86	0.80	**0.55 s**	**0.49 s**	2	4	4	4
IV.8 (F22)	8.3	3.9	18.2	6.5	4.7	23.4	**0.79 s**	**0.69 s**	1.02 **s**	1.26	0.82 **s**	0.76 **s**	1	3	2	4
IV.9 (F19)	3.8	3.9	25.9	16.2	4.2	25.3	**0.70 s**	**0.76 s**	**0.64**	**0.79**	**0.56 s**	**0.62**	1	4	2	4
IV.10 (M17)	4.7	5.5	21	0	0	0	0.87 **s**	0.96 **s**	**0.51**	0.92	**0.62**	**0.62**	1	4	2	4
V.1 (M5)	5.9	4.2	10.1	4.9	3.8	19.7	**0.81**	**0.81**								
V.5 (F14)	6.7	4.7	22	0	0	0	**0.72 s**	**0.71 s**	0.85	1.07	**0.66 s**	**0.62 s**	1	1	2	4
V.6 (M12)	3.3	3.5	20	0	0	0	0.86 **s**	0.89 **s**	**0.76**	**0.67**	**0.65**	0.70	1	1	4	4
V.7 (M10)	5.1	3.5	21	0	0	0	**0.66 s**	0.85 **s**	**0.80**	**0.76**	**0.62**	**0.66 s**	1	1	4	4
V.12 (M5)	3.6	3.1	21.2	8.2	2.9	24.1	**0.78**	0.86								
Unaffected																
IV.4 (F40)							1.05	1.26	0.67	1.00	0.84	0.60	1	1	1	1
V.2 (F3)							0.83	0.88 *****					1	1	1	1

s= catch-up saccade compensating for vestibulo-ocular reflex deficit. EO = Eyes Open; EC = Eyes Closed.

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

Gülden Akdal, Koray Koçoglu, Elçin Bora, et al.

Neurol Clin Pract published online September 15, 2020
DOI 10.1212/CPJ.0000000000000930