Confounding clinical presentation and different disease progression in CMT4B1

Raquel Guimarães-Costa, Rocio-Nur Villar-Quiles, Philippe Latour, Guilhem Sole, Isabelle Husson, Arnaud Lacour, Sarah Leonard-Louis, Tanya Stojkovic

Title: Confounding clinical presentation and different disease progression in CMT4B1

Raquel Guimarães-Costa[1#], Rocio-Nur Villar-Quiles[1#], Philippe Latour[2], Guilhem Sole[3], Isabelle Husson[4], Arnaud Lacour[5], Sarah Leonard-Louis[1], Tanya Stojkovic[1]

Author names and affiliations:

[1] APHP, Centre de référence des maladies neuromusculaires, Institut de myologie, Sorbonne Université, APHP, Hôpital Pitié-Salpêtrière; Paris, France

[2] UF de neurogénétique héréditaire (UF 34427), Centre de Biologie et Pathologie Est - Service de Biochimie Biologie Moléculaire Grande Est; Hospices Civils, Lyon, France

[3] Service de neurologie, CHU Bordeaux, Bordeaux, France

[4] Service de neuropediatrie, APHP, Hôpital Robert Debré, Paris, France

[5] Service de neurologie, CHU Lille, Lille, France

# Co-first authors with equal contribution

Corresponding author:

Tanya Stojkovic.

Centre de Référence des maladies neuromusculaires Nord / Est / Ile-de-France

Groupe Hospitalier Pitié-Salpêtrière

47-83 boulevard de l'Hôpital

75651 Paris Cedex 13

E-mail address: tanya.stojkovic@aphp.fr

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest: None

Abstract

We report seven Charcot-Marie-Tooth 4B1 (CMT4B1) patients from four families with distinctive features, presenting with severe distal weakness and cranial nerve involvement. Patient from family 1 presented with congenital *varus* foot deformity, progressive distal and proximal weakness leading to loss of ambulation at 14 years, bilateral facial palsy and prominent bulbar involvement. In three siblings from family 2, still ambulant in the second decade, neuropathy was associated with marked sweating and Arnold-Chiari syndrome. Patient from family 3, wheelchair-bound by 17 years, suffered from recurrent intestinal occlusion due to a mesenteric malformation. Patients from family 4, wheelchair-bound from age 6 years, were first diagnosed with type 1 Usher syndrome with congenital deafness and retinitis pigmentosa. CMT4B1 diagnosis was based upon suggestive clinical features and confirmed by the presence of recessive mutations in the *MTMR2* gene. Our results expand the genetic and phenotypic spectrum of CMT4B1, which may include autonomic system involvement.

Keywords: Neuropathy, CMT, MTMR2 gene, Usher, Chiari Syndrome, Bulbar and facial weakness

1. Introduction

Charcot-Marie-Tooth (CMT) is the most frequent hereditary sensory and motor neuropathy (10-30/1000,000)[1]. Genetic testing is guided by clinical signs, nerve conduction studies and mode of inheritance.

Autosomal recessive demyelinating forms (CMT4) are related to mutations in at least 12 genes[2]. Mutations in myotubularin-related protein 2 (*MTMR2*) gene[3,4], causing CMT4B1, are very rare, with an estimated frequency of less than 0.2% among CMT patients[5].

MTMR2 protein is a phospholipid phosphatase that participates in the control of longitudinal myelin growth[6]. *MTMR2* gene has a PH-GRAM domain (Plecktrim homology, glucotransferase, Rab-like GTPase activator and myotubularin), responsible for binding phosphoinositides, a PTP domain (homology with protein tyrosine phosphatase), a coiled-coil (CC) domain responsible for homo- and heterodimerisation with other members of the family, and a PDS-95-Dlg-ZO-1 (PDZ-BD) binding domain at the C-terminus[7].

MTMR2 mutations cause an early onset demyelinating neuropathy with loss of ambulation around the second or third decade[8–14].

We report seven patients, in which the classical clinical presentation was associated with distinctive features, posing a diagnostic challenge.

2. Case report

2.1 Probands

We describe seven patients (4 women, 3 men) from four families.

Family 1 has never been reported. Families 2,3 and 4 were included in a recent multicentric CMT4B study[13], but details of clinical presentation and disease progression were not reported, nor was there a discussion of relevant genetic features.

Informed consent was obtained from all patients. Motor deficit was evaluated using the MRC scale. Charcot-Marie-Tooth neuropathy score (CMTNS) was calculated to characterize disability and progression of the disease[15]. Nerve conduction studies were performed using standard techniques[16].

2.2. Families

Clinical data are reported in Table 1.

2.2.1. Family 1

Subject II_1 is the only affected member of a Georgian non-consanguineous family(Fig.1A). She had a congenital forefoot *varus* deformation requiring surgical correction at 2 years. Although she started to walk at 11 months, she had difficulties in running and frequent falls. Distal and subsequent proximal motor weakness lead to loss of ambulation at the age of 14 years. Diagnosis of a demyelinating hereditary neuropathy was made around the age of 16 but genetic confirmation was obtained at 32. Examination showed bilateral facial palsy, hoarseness, bilateral palate palsy, abolished pharyngeal reflexes, retrognathia severe global wasting and weakness, areflexia and limb contractures affecting finger flexors, psoas and hamstring muscles. Severe restrictive respiratory insufficiency (FVC 27% of predicted values) was diagnosed at 32 years, prompting the initiation of non-invasive nocturnal ventilation.

2.2.2. Family 2

The three patients from this non-consanguineous French family presented with delayed motor milestones and falls since early infancy. Diagnosis of a demyelinating hereditary neuropathy was made around the age of 2 years but genetic confirmation was obtained much later.

They presented diffuse contractures, areflexia and asymmetric but bilateral facial palsy. Subject II_3 also presented with hoarse voice without swallowing difficulties. All have predominantly distal weakness(Table 1). Walking unaided was possible, though unstable and with a Romberg sign. They had no cognitive impairment and no visual or auditory symptoms. Increased whole body sweating was present in all three patients without other signs of dysautonomia.

Spine and cerebral magnetic resonance imaging (MRI) were performed in patients and their parents. An Arnold-Chiari Chiari type 1 malformation was detected in all patients, associated with cervical (C5-C7 in II_4, C5-C6 in II_5) or dorsolumbar (D6-T1 in II_3) syringomyelia(Fig.1-B,C). The father had an isolated and asymptomatic syringomyelia. Subjects II_3 and II_5 underwent surgical correction for this Arnold-Chiari malformation since it was considered responsible for the worsening of motor function.

2.2.3. Family 3

Subject II_1 is the only affected member of a French non-consanguineous family. He presented with delayed motor development and frequent falls from the age of 2 years, leading to the diagnosis of severe demyelinating sensorimotor neuropathy by

the age of 8. He became wheelchair-bound by the age of 17 years and required assistance for transfers at about 21 years.

Examination showed bilateral facial palsy, hoarseness, impaired tongue mobility, osteoarticular deformations and dysmorphic features (prognathism, prominent lips, large forehead, bilateral scapular winging, high-implanted hair and slight testicular atrophy).

Severe restrictive respiratory insufficiency (FVC 25%) was diagnosed at 25 years old, requiring non-invasive nocturnal ventilation.

A nerve biopsy showed extreme fibre loss with only unmyelinated fibres left.

After the age of 30 years, he developed unexplained episodes of epigastric pain, with nausea and diarrhoea consistent with bowel occlusions and lost 15 kg. A diagnosis of incomplete common mesentery was made.

2.2.4. Family 4

Patients II_1 and II_3 were born to consanguineous parents from Mauritius.

Ocular examinations showed retinitis pigmentosa and narrowed visual fields.

Patients had delayed motor milestones including neck and trunk control. They had also delay in language development and began to vocalize at age 2 years. Profound congenital deafness, vestibular dysfunction and retinitis pigmentosa were detected in both children leading to the diagnosis of Usher syndrome type 1. Patient II_1 begun walking at 3 years, used wheelchair at the age of 6 and wheelchair-bound at 19. Patient II_3 walked at 12 months, required a wheelchair from the age of 6 years and became wheelchair-bound at 12 after a tibia fracture.

The pattern of contractures, cranial nerve involvement and muscle strength in these two patients are reported in Table 1. Sensory examination showed apallesthesia in

151 the lower limbs and hypopallesthesia in the upper limbs. In patient II_1 vocalizations
152 and expiratory sounds were hoarse and examination confirmed tongue and palate
153 hypomobility and probable vocal cord paralysis. Both sisters presented swallowing
154 problems since childhood.
155 Subject II_1 was diagnosed at 22 years with restrictive respiratory insufficiency (FVC
156 32%) associated with severe obstructive sleep apnoea syndrome, requiring nocturnal
157 non-invasive ventilation. Subject II_2's latest is FVC 43%, prompting the initiation of
158 non-invasive ventilation.
159 Subject II_1 had a nerve and muscle biopsy, which revealed major muscle atrophy and
160 a severe rarefaction of myelinated fibres. Thickened and redundant myelin loops
161 were observed under electron microscopy(Fig.1-E,F).
162 A brain MRI was performed in these two siblings, showing a bilateral and symmetrical
163 linear T2 hyperintensity in the posterior pons.
164
165 ***2.3. Electroneuromyography***
166 Motor nerve conduction velocities (MNCV) were significantly slowed in family 2
167 suggesting a demyelinating neuropathy(Table 2). Distal motor latencies were
168 prolonged. Compound muscle action potentials were either severely reduced or not
169 obtained. MNCV were not recordable in families 1, 3 and 4, who were severely
170 disabled. Sensory amplitudes were abolished in families 1 and 3 and diminished in
171 family 2. Electromyography disclosed chronic denervation signs in all four limbs.
172
173 ***2.4. Genetic analysis of MTMR2 gene***
174 Complete Sanger sequencing of the *MTMR2* coding sequence was performed
175 (reference transcript NM_016156). The Genome Aggregation Database (GnomAD:

https://gnomad.broadinstitute.org) was interrogated for known variants in the general population. The predictive analysis '*in silico*' for the missense variant p.Thr537Ile was done using the SIFT and PolyPhen-2 programs.

Patient II_1 from family 1 bears a homozygous variant c.1633_1636dup (p.Ser546Lysfs*9). This frameshift variant, absent on GnomAD database, results in a premature stop codon leading to a truncated protein devoid of the CC and the PDZ-BD domains.

Family 2 bears two composite heterozygous variants: c.1375C>T/c.1610C>T (p.Arg459*/p.Thr537Ile). Thr537 is located in the PTP domain and is highly conserved. Predictions for a substitution p.Thr537Ile, absent on GnomAD database, were deleterious (SIFT, score 0.01) and probably damaging (PolyPhen-2, score 0.996).

Patient from family 3 had a composite heterozygous pattern with two premature stop codon variants, c.617G>A and c.1749G>A (p.Trp206*/p.Trp583*), the first one being inherited from the patient's mother.

In family 4, the homozygous stop variant c.1030C>T (p.Gln344*) was detected in affected siblings in addition to a homozygous *MYO7A* gene mutation (c.5617C>T, p.Arg1873Trp) related to the Usher syndrome type 1. A karyotype analysis was performed in subject II_1 since *MYO7A* and *MTMR2* are in close chromosomic regions (*MYO7A* at 11q13.5; *MTMR2* at 11q22). There was no deletion of this region. Both parents carry the *MYO7A variant* and a heterozygous p.Gln344* *MTMR2* variant.

3. Discussion

In addition to the motor and sensory peripheral nerve involvement leading to severe weakness and frequent loss of ambulation, the clinical spectrum of CMT4B1 encompasses cranial nerve involvement, claw hand and respiratory insufficiency. Moreover, CMT4B1 is associated with a large genetic and phenotypic variability.

Although the phenotype was highly evocative in patient II_1 from family 1, CMT4B1 diagnosis was delayed and made after the diagnosis of Arnold-Chiari syndrome and Usher syndrome, in families 2 and 4. In family 3 additional dysmorphic features, such as macroglossia, large forehead and high-implanted hair have not been previously described in association with CMT4B1.

Two cases of CMT1A associated with either an asymptomatic C2-C4[17] or a T11-L1 syringomyelia and urinary disorders[18] have been reported to date. Furthermore, syringomyelia may be due to mutations in genes such as *GDF6* (8q22),*GDF3* (12p13)[19] or *MEOX1* genes[20] related to Klippel-Feil syndrome. To our knowledge, syringomyelia has not been reported in CMT4B1 patients.

Strikingly, affected members of family 2 have increased whole body sweating. We postulate that there might be an involvement of thin myelinated Aδ fibres, resulting in abnormal sudation.

Patient from family 3 presented incomplete common mesentery, a defect of mesentery rotation rarely reported in adults. Recently, a case of CMT2S (*IGHMBP2* mutation) associated with severe gastrointestinal dysautonomia was described[21]. To our knowledge, the only genetic syndromes manifesting with this intestinal abnormality are Wolf-Hirschhorn and Pitt-Rogers-Danks syndromes, associated with

deletions in chromosome 4 and severe cognitive impairment, which does not correspond to our patient's presentation[22,23]. An alternative explanation for the gastrointestinal manifestations and increased sweating is that neuro-vegetative manifestations could be associated with severe forms of CMT and might be independent of the CMT mutation itself. Autonomic system involvement in CMT patients has already been postulated in CMT related to *MPZ* mutations[24]. Finally, the occurrence of two genetic disorders, Usher syndrome and CMT4B1, is conceivable in the context of consanguinity.

Our results also indicate that respiratory involvement may correlate with disease severity. Patients with early loss of ambulation present restrictive respiratory failure with low FVC values. Conversely, ambulatory patients in adulthood have preserved respiratory function.

Most *MTMR2* variants reported to date represent loss-of function alleles (nonsense or frameshift variants) leading to premature truncated proteins which would be degraded[13]. So far, no clear genotype-phenotype correlations have been found for CMT4B1[13]. Interestingly, for CMT4B3 due to *MTMR5/SBF1* mutations, the mutation type and localization correlates with disease severity. As such, certain missense mutations are predicted to be benign with a mild impact on MTMR5 protein function[13,25]. Missense mutations have been associated with a milder course in other forms of recessive CMT such as CMT4A due to *GDAP1* mutations[26].

Some authors have suggested that disease severity in CMT4B1 could be related to the mutation localization. Variants affecting either PH-GRAM or the PTP domains would result in loss of the phosphatase activity and a more severe phenotype[8,27]. Indeed, affected members of family 4 who lost ambulation during the first decade carry a stop variant affecting the PTP domain. A homozygous variant leading to a

similarly truncated protein is carried by the patient from family 1, wheelchair-bound at age 14 years. Regarding family 3, two different compound heterozygous nonsense variants were found: p.Trp206*, located in the PTP domain abolishing the enzymatic activity of the protein, and p.Trp583*. The latter preserves the PTP and PH-GRAM domains, but affects the CC and PDZ-BD domains and has been previously associated with a more severe presentation[28]. Interestingly, this latter report also concerns a French patient, which could lead us to hypothesize a possible recurrence of this variant in the French population.

In family 2, a milder disease progression was observed in the three siblings carrying a stop variant (p.Arg459*) combined with a missense variant p.Thr537Ile in the phosphatase domain, potentially preserving a partial residual catalytic activity. There are few CMTB1 milder cases reported[8,11]. Indeed, a patient with disease onset at 13 years still ambulant at 34 carried a homozygous frameshift variant in the extreme C-terminus of *MTMR2* gene (p.Arg628fs*18)[13,11], between the CC and PDZ-BD domains, affecting only PDZ-BD function, and preserving the domains responsible for catalytic activity. Thus, a partially preserved function of MTMR2 protein could explain milder phenotypes.

CMT4B1 diagnosis can be challenging in the presence of other medical conditions, especially in consanguineous families. However, all these families shared some common features i: severe distal and proximal weakness, ii) facial and bulbar palsy, contractures and ii) demyelinating neuropathy (evidenced on ENMG or nerve biopsy) suggesting a *MTMR2* mutation. The additional phenotypical findings present in our patients, especially the dysautonomic symptoms, could also be part of the CMT4B1 clinical spectrum.

Acknowledgements

We gratefully acknowledge the families for their kind cooperation and Dr Dubourg for her collaboration in this study.

References

[1] Vallat JM, Mathis S, Funalot B. The various Charcot-Marie-Tooth diseases. Curr Opin Neurol 2013;26:473–80. doi:10.1097/WCO.0b013e328364c04b.

[2] Pipis M, Rossor AM, Laura M, Reilly MM. Next-generation sequencing in Charcot–Marie–Tooth disease: opportunities and challenges. Nat Rev Neurol 2019;15:644–56. doi:10.1038/s41582-019-0254-5.

[3] Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, et al. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. Nat Genet 2000;25:17–9. doi:10.1038/75542.

[4] Gambardella A, Muglia M, Quattrone A. Hereditary demyelinating neuropathy of infancy: a genetically complex syndrome. Brain 1997;120 (Pt 11):2113–5. doi:10.1093/brain/120.11.2113.

[5] Murphy SM, Laura M, Fawcett K, Pandraud A, Liu Y-T, Davidson GL, et al. Charcot-Marie-Tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry 2012;83:706–10. doi:10.1136/jnnp-2012-302451.

[6] Vaccari I, Dina G, Tronchère H, Kaufman E, Chicanne G, Cerri F, et al. Genetic interaction between MTMR2 and FIG4 phospholipid phosphatases involved in Charcot-Marie-Tooth neuropathies. PLoS Genet 2011;7:e1002319. doi:10.1371/journal.pgen.1002319.

[7] Previtali SC, Quattrini A, Bolino A. Charcot-Marie-Tooth type 4B demyelinating neuropathy: deciphering the role of MTMR phosphatases. Expert Rev Mol Med 2007;9:1–16. doi:10.1017/S1462399407000439.

[8] Houlden H. Mutations in the 5' region of the myotubularin-related protein 2 (MTMR2) gene in autosomal recessive hereditary neuropathy with focally folded myelin. Brain 2001;124:907–15. doi:10.1093/brain/124.5.907.

[9] Nelis E, Erdem S, Tan E, Löfgren A, Ceuterick C, De Jonghe P, et al. A novel homozygous missense mutation in the myotubularin-related protein 2 gene associated with recessive Charcot-Marie-Tooth disease with irregularly folded myelin sheaths. Neuromuscul Disord 2002;12:869–73. doi:10.1016/S0960-8966(02)00046-9.

[10] Nouioua S, Hamadouche T, Funalot B, Bernard R, Bellatache N, Bouderba R, et al. Novel mutations in the PRX and the MTMR2 genes are responsible for unusual Charcot-Marie-Tooth disease phenotypes. Neuromuscul Disord NMD 2011;21:543–50. doi:10.1016/j.nmd.2011.04.013.

[11] Murakami T, Kutoku Y, Nishimura H, Hayashi M, Abe A, Hayasaka K, et al. Mild phenotype of Charcot-Marie-Tooth disease type 4B1. J Neurol Sci 2013;334:176–9. doi:10.1016/j.jns.2013.08.001.

[12] Zambon AA, Natali Sora MG, Cantarella G, Cerri F, Quattrini A, Comi G, et al. Vocal cord paralysis in Charcot–Marie–Tooth type 4b1 disease associated with a novel mutation in the myotubularin-related protein 2 gene: A case report and review of the literature. Neuromuscul Disord 2017;27:487–91. doi:10.1016/j.nmd.2017.01.006.

[13] Pareyson D, Stojkovic T, Reilly MM, Leonard-Louis S, Laurà M, Blake J, et al. A multicentre retrospective study of Charcot-Marie-Tooth disease type 4B (CMT4B) due to mutations in Myotubularin-related proteins (MTMRs). Ann Neurol 2019. doi:10.1002/ana.25500.

[14] Wang H, Bayram AK, Sprute R, Ozdemir O, Cooper E, Pergande M, et al. Genotype-phenotype correlations in charcot-marie-tooth disease due to mtmr2 mutations and implications in membrane trafficking. Front Neurosci 2019;13. doi:10.3389/fnins.2019.00974.

[15] Shy ME, Blake J, Krajewski K, Fuerst DR, Laura M, Hahn AF, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. Neurology 2005;64:1209–14. doi:10.1212/01.WNL.0000156517.00615.A3.

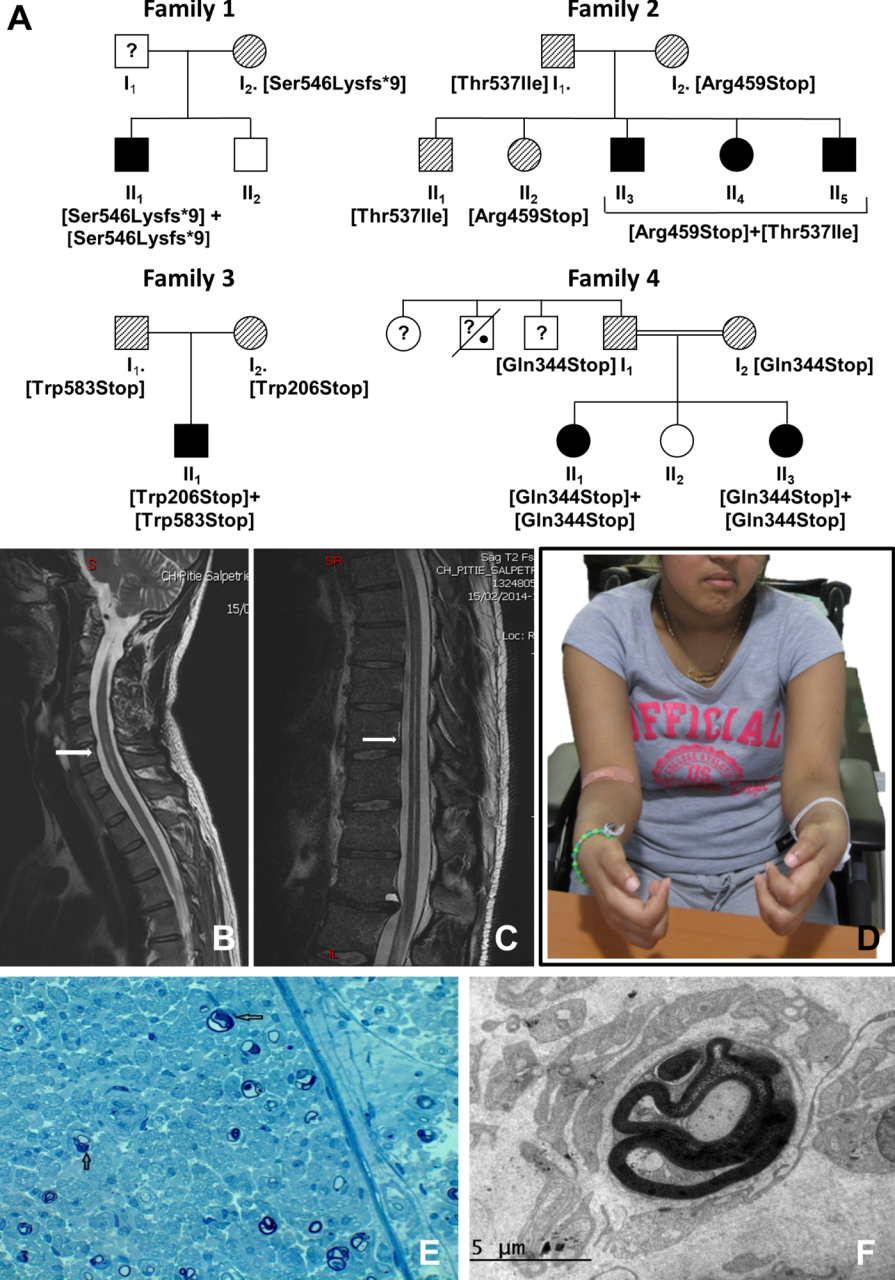
[16] Tankisi H, Pugdahl K, Beniczky S, Andersen H, Fuglsang-Frederiksen A. Evidence-based recommendations for examination and diagnostic strategies of polyneuropathy electrodiagnosis. Clin Neurophysiol Pract 2019;4:214–22. doi:10.1016/j.cnp.2019.10.005.
[17] Bütefisch C, Gutmann L, Gutmann L. Compression of spinal cord and cauda equina in Charcot-Marie-Tooth disease type 1A. Neurology 1999;52:890–1. doi:10.1212/wnl.52.4.890.
[18] Lassalle A, Durufle A, Le Tallec H, Laboute E, Kerdoncuff V, Plassat R, et al. [Charcot-Marie-Tooth disease associated with syringomyelia revealed by urinary disorders]. Ann Readapt Med Phys Rev Sci La Soc Fr Reeduc Fonct Readapt Med Phys 2005;48:146–9. doi:10.1016/j.annrmp.2004.09.007.
[19] Markunas CA, Soldano K, Dunlap K, Cope H, Asiimwe E, Stajich J, et al. Stratified whole genome linkage analysis of Chiari type I malformation implicates known Klippel-Feil syndrome genes as putative disease candidates. PLoS One 2013;8:e61521. doi:10.1371/journal.pone.0061521.
[20] Bayrakli F, Guclu B, Yakicier C, Balaban H, Kartal U, Erguner B, et al. Mutation in MEOX1 gene causes a recessive Klippel-Feil syndrome subtype. BMC Genet 2013;14:95. doi:10.1186/1471-2156-14-95.
[21] Tomaselli PJ, Horga A, Rossor AM, Jaunmuktane Z, Cortese A, Blake JC, et al. IGHMBP2 mutation associated with organ-specific autonomic dysfunction. Neuromuscul Disord NMD 2018. doi:10.1016/j.nmd.2018.08.010.
[22] Bergemann AD, Cole F, Hirschhorn K. The etiology of Wolf-Hirschhorn syndrome. Trends Genet 2005;21:188–95. doi:10.1016/j.tig.2005.01.008.
[23] Sukarova-Angelovska E, Kocova M, Sabolich V, Palcevska S, Angelkova N. Phenotypic variations in wolf-hirschhorn syndrome. Balkan J Med Genet 2014;17:23–30. doi:10.2478/bjmg-2014-0021.
[24] Stojkovic T, de Seze J, Dubourg O, Arne-Bes MC, Tardieu S, Hache JC, et al. Autonomic and respiratory dysfunction in Charcot-Marie-Tooth disease due to Thr124Met mutation in the myelin protein zero gene. Clin Neurophysiol Off J Int Fed Clin Neurophysiol 2003;114:1609–14.
[25] Nakhro K, Park JM, Hong Y Bin, Park JH, Nam SH, Yoon BR, et al. SET binding factor 1 (SBF1) mutation causes Charcot-Marie-Tooth disease type 4B3. Neurology 2013;81:165–73. doi:10.1212/WNL.0b013e31829a3421.
[26] Cassereau J, Chevrollier A, Bonneau D, Verny C, Procaccio V, Reynier P, et al. A locus-specific database for mutations in GDAP1 allows analysis of genotype-phenotype correlations in Charcot-Marie-Tooth diseases type 4A and 2K. Orphanet J Rare Dis 2011;6:87. doi:10.1186/1750-1172-6-87.
[27] Luigetti M, Bolino A, Scarlino S, Sabatelli M. A novel homozygous mutation in the MTMR2 gene in two siblings with “hypermyelinating neuropathy”. J Peripher Nerv Syst 2013;18:192–4. doi:10.1111/jns5.12019.
[28] Verny C, Ravisé N, Leutenegger A-L, Pouplard F, Dubourg O, Tardieu S, et al. Coincidence of two genetic forms of Charcot-Marie-Tooth disease in a single family. Neurology 2004;63:1527–9. doi:10.1212/01.wnl.0000142082.65144.ee.

Figure legends

Figure 1. A: Family pedigrees. Black filled areas: affected individual; hatch symbol: heterozygous individual; ?: unknown genetic, clinically asymptomatic; crossed white symbol with black point: dead during infancy, epilepsy. **B-C: Spine MRI from Family 2, subjects I_1 (B) and II_1 (C):** syringomyelia. **D: Clinical findings in Family 4, subject II_1:** claw hands with distal motor weakness and elbows contractures. **E-F: Muscle biopsy findings.** Sural nerve biopsy from subject II_1 (Family 4). E: Semi-thin section (thionin blue staining) shows a severe loss of myelinated fibers. Note the presence of myelin outfoldings (arrows). F: Electronic microscopy revealed focally folded myelin.

Table 1: Summarized clinical features. A: absent; D: diminished; FVC: Forced vital capacity; LL: lower limbs; N: no; NA: not available; NIV: non-invasive ventilation; NI: normal; NR: not reliable (due to communication difficulties); P: present, UL: upper limbs; Y: yes. #: mute (Uscher syndrome).

Table 2: Electrophysiological data. EMG: electroneuromyogram; MCV: Motor Conduction Velocity (meters/second); ms: milliseconds; mV: millivolts; NA: not available; NO: not obtained; µV: microvolts; SNCV: Sensory nerve conduction velocity: Median nerve (palm stimulation): orthodromic stimulation.


A
Family 1
?
I1
I2. [Ser546Lysfs*9]
II1
[Ser546Lysfs*9] +
[Ser546Lysfs*9]
II2
Family 2
[Thr537Ile] I1.
I2. [Arg459Stop]
II1
[Thr537Ile]
II2
[Arg459Stop]
II3
II4
II5
[Arg459Stop]+[Thr537Ile]
Family 3
I1.
[Trp583Stop]
I2.
[Trp206Stop]
II1
[Trp206Stop]+
[Trp583Stop]
Family 4
?
?
?
[Gln344Stop] I1
I2 [Gln344Stop]
II1
[Gln344Stop]+
[Gln344Stop]
II2
II3
[Gln344Stop]+
[Gln344Stop]
B
C
D
E
5 µm
F

Family		1	2			3	4	
Subject		II_1	II_3	II_4	II_5	II_1	II_1	II_3
cDNA change		[1633_1636dup] homozygous	[1375C>T]+ [1610C>T]			[617G>A]+ [1749G>A]	[1030C>T] homozygous	
Protein change		[Ser546Lysfs*9]	[Arg459*]+[Thr537Ile]			[Trp206*]+ [Trp583*]	[Gln344*]	
Gait acquisition (months)		11	16	16	16	NA	36	12
Age at last evaluation		32	24	22	15	32	22	17
Facial diplegia (Y/N)		Y	Y	Y	NA	Y	Y	Y
Dysphagia (Y/N)		Y	N	N	N	Y	Y	Y
Other cranial nerve involvement (Y/N)		Y	N	N	N	Y	Y	Y
Hoarse voice (Y/N)		Y	Y	N	N	Y	Y, Mute#	Y, Mute#
Foot deformities		*Equinovarus*	*Cavus*	*Planus*	*Planus*	*Cavus*	*Cavus*	*Planus*
Spine deformities		N	Scoliosis	NA	NA	Scoliosis and hyperlordosis	Scoliosis	NA
Contractures								
UL	Proximal	N	Y	Y	Y	N	N	N
	Distal (claw hands)	Y	Y	Y	Y	Y	Y	Y
LL	Proximal	Y	Y	Y	Y	Y	Y	Y
	Distal	Y	N	N	N	N	Y	Y
Deep tendon reflexes (A/P)		A	A	A	A	A	A	A
Motor Testing (MRC)								
UL	Proximal	2	5	5	5	3	4	NA
	Distal	0	1	2	2	0	1	NA
LL	Proximal	0	2	3	5	1	2	NA
	Distal	0	0	0	2	0	0	NA
Sensory Examination								
Pin-prick		D	D	D	D	D	NR	NR
Hypopallesthesia		Y	Y	Y	Y	Y	NR	NR
CMTES/CMTNS		16/32	15/23	12/20	10/16	16/32	16/32	19/NA
Loss of ambulation (years)		14	Ambulatory	Ambulatory	Ambulatory	11	6	6
Respiratory involvement								
FVC (%)		27%	95%	NA	NA	25%	32%	43%
NIV (Y/N), age (years)		Y, 32	N	N	N	Y, 28	Y, 22	Y, 17
Cardiac involvement (Y/N)		N	Y, moderate aortic insufficiency	N	N	N	N	N
Other features		Bilateral palatal palsy, retrognathia	Syringomyelia and Chiari Malformation Whole body hyperhidrosis			Common incomplete mesentery, Facial dysmorphism	Bilateral ptosis, bilateral scapular winging. Type 1 Usher Syndrome.	Bilateral ptosis. Type 1 Usher Syndrome

Table 1: Summarized clinical features. A: absent; D: diminished; FVC: Forced vital capacity; LL: lower limbs; N: no; NA: not available; NIV: non-invasive ventilation; Nl: normal; NR: not reliable (due to communication difficulties); P: present, UL: upper limbs; Y: yes. #: mute (Usher syndrome).

Family			1	2			3	4	Normal values
Subject			II_1	II_3	II_4	II_5	II_1	II_1	
Age (years)			32	4	20	12	8	21	
Motor Nerves (*recorded muscle*)	Peroneal (t*ibialis anterior*)	Distal Latency (ms)	NO	10,8	NO	4,5	NO	NO	≤5
		Amplitude (mV)	NO	1,5	NO	3	NO	NO	≥3
		MCV (m/s)	NO	21	NO	25	NO	NO	≥42
	Median (*abductor pollicis brevis*)	Distal Latency (ms)	NO	3,7	3.9	2,9	NO	NO	≤3,7
		Amplitude (mV)	NO	2,4	1,5	2,6	NO	NO	≥6
		MCV (m/s)	NO	25	21	34	NO	NO	≥48
	Facial (*orbicularis oculi*)	Distal Latency (ms)	NO	NA	NA	NA	NA	6,5	≤3,1
		Amplitude (mV)	NO	NA	NA	NA	NA	0,14	≥1
	Hypoglossal (*genioglossus*)	Distal Latency (ms)	NO	NA	NA	NA	NA	5,4	≤2,2
		Amplitude (mV)	NO	NA	NA	NA	NA	0,26	≥3
Sensory Nerves	Sural	Amplitude (µV)	NO	NA	NO	NA	NO	NO	≥10
		SNCV (m/s)	NO	NA	NO	NA	NO	NO	≥40
	Median	Amplitude (µV)	NO	12	5,1	20	NO	NO	≥15
		SNCV (m/s)	NO	27	27	41	NO	NO	≥45

Table 2: Electrophysiological data. EMG: electroneuromyogram; MCV: Motor Conduction Velocity (meters/second); ms: milliseconds; mV: millivolts; NA: not available; NO: not obtained; µV: microvolts**;** SNCV: Sensory nerve conduction velocity: Median nerve (palm stimulation): orthodromic stimulation.