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► **To cite this version:**

Raquel Guimarães-Costa, Rocío-Nur Villar-Quiles, Philippe Latour, Guilhem Sole, Isabelle Husson, et al.. Confounding clinical presentation and different disease progression in CMT4B1. *Neuromuscular Disorders*, 2020, 30 (7), pp.576-582. 10.1016/j.nmd.2020.05.003 . hal-02946406

HAL Id: hal-02946406

<https://hal.sorbonne-universite.fr/hal-02946406v1>

Submitted on 22 Aug 2022

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1 **Title:** Confounding clinical presentation and different disease progression in
2 CMT4B1

3
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26 **Funding:** This research did not receive any specific grant from funding agencies in
27 the public, commercial, or not-for-profit sectors.

28 **Declarations of interest:** None

29

30

31 **Abstract**

32 We report seven Charcot-Marie-Tooth 4B1 (CMT4B1) patients from four families with
33 distinctive features, presenting with severe distal weakness and cranial nerve
34 involvement. Patient from family 1 presented with congenital *varus* foot deformity,
35 progressive distal and proximal weakness leading to loss of ambulation at 14 years,
36 bilateral facial palsy and prominent bulbar involvement. In three siblings from family
37 2, still ambulant in the second decade, neuropathy was associated with marked
38 sweating and Arnold-Chiari syndrome. Patient from family 3, wheelchair-bound by 17
39 years, suffered from recurrent intestinal occlusion due to a mesenteric malformation.
40 Patients from family 4, wheelchair-bound from age 6 years, were first diagnosed with
41 type 1 Usher syndrome with congenital deafness and retinitis pigmentosa. CMT4B1
42 diagnosis was based upon suggestive clinical features and confirmed by the
43 presence of recessive mutations in the *MTMR2* gene. Our results expand the genetic
44 and phenotypic spectrum of CMT4B1, which may include autonomic system
45 involvement.

46

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

49

50

51

52

53 **1. Introduction**

54

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

58 Autosomal recessive demyelinating forms (CMT4) are related to mutations in at least
59 12 genes[2]. Mutations in myotubularin-related protein 2 (*MTMR2*) gene[3,4], causing
60 CMT4B1, are very rare, with an estimated frequency of less than 0.2% among CMT
61 patients[5].

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

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

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

73

74 **2. Case report**

75 **2.1 Probands**

76

77 We describe seven patients (4 women, 3 men) from four families.
78 Family 1 has never been reported. Families 2,3 and 4 were included in a recent
79 multicentric CMT4B study[13], but details of clinical presentation and disease
80 progression were not reported, nor was there a discussion of relevant genetic
81 features.

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

86

87 **2.2. Families**

88 Clinical data are reported in Table 1.

89

90 **2.2.1. Family 1**

91 Subject II₁ is the only affected member of a Georgian non-consanguineous
92 family(Fig.1A). She had a congenital forefoot *varus* deformation requiring surgical
93 correction at 2 years. Although she started to walk at 11 months, she had difficulties
94 in running and frequent falls. Distal and subsequent proximal motor weakness lead to
95 loss of ambulation at the age of 14 years. Diagnosis of a demyelinating hereditary
96 neuropathy was made around the age of 16 but genetic confirmation was obtained at
97 32. Examination showed bilateral facial palsy, hoarseness, bilateral palate palsy,
98 abolished pharyngeal reflexes, retrognathia severe global wasting and weakness,
99 areflexia and limb contractures affecting finger flexors, psoas and hamstring muscles.
100 Severe restrictive respiratory insufficiency (FVC 27% of predicted values) was
101 diagnosed at 32 years, prompting the initiation of non-invasive nocturnal ventilation.

102

103 **2.2.2. Family 2**

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

108 They presented diffuse contractures, areflexia and asymmetric but bilateral facial
109 palsy. Subject II₃ also presented with hoarse voice without swallowing difficulties. All
110 have predominantly distal weakness(Table 1). Walking unaided was possible, though
111 unstable and with a Romberg sign. They had no cognitive impairment and no visual
112 or auditory symptoms. Increased whole body sweating was present in all three
113 patients without other signs of dysautonomia.

114 Spine and cerebral magnetic resonance imaging (MRI) were performed in patients
115 and their parents. An Arnold-Chiari Chiari type 1 malformation was detected in all
116 patients, associated with cervical (C5-C7 in II₄, C5-C6 in II₅) or dorsolumbar (D6-T1
117 in II₃) syringomyelia(Fig.1-B,C). The father had an isolated and asymptomatic
118 syringomyelia. Subjects II₃ and II₅ underwent surgical correction for this Arnold-Chiari
119 malformation since it was considered responsible for the worsening of motor
120 function.

121

122 **2.2.3. Family 3**

123 Subject II₁ is the only affected member of a French non-consanguineous family. He
124 presented with delayed motor development and frequent falls from the age of 2
125 years, leading to the diagnosis of severe demyelinating sensorimotor neuropathy by

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

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

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

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

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

138

139 **2.2.4. Family 4**

140 Patients II₁ and II₃ were born to consanguineous parents from Mauritius.

141 Ocular examinations showed retinitis pigmentosa and narrowed visual fields.

142 Patients had delayed motor milestones including neck and trunk control. They had
143 also delay in language development and began to vocalize at age 2 years. Profound
144 congenital deafness, vestibular dysfunction and retinitis pigmentosa were detected in
145 both children leading to the diagnosis of Usher syndrome type 1. Patient II₁ began
146 walking at 3 years, used wheelchair at the age of 6 and wheelchair-bound at 19.

147 Patient II₃ walked at 12 months, required a wheelchair from the age of 6 years and
148 became wheelchair-bound at 12 after a tibia fracture.

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

151 the lower limbs and hypopallesthesia in the upper limbs. In patient II₁ 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 II₁ 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 II₂'s latest is FVC 43%, prompting the initiation of
158 non-invasive ventilation.

159 Subject II₁ 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:

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

179 Patient II₁ from family 1 bears a homozygous variant c.1633_1636dup
180 (p.Ser546Lysfs*9). This frameshift variant, absent on GnomAD database, results in a
181 premature stop codon leading to a truncated protein devoid of the CC and the PDZ-
182 BD domains.

183 Family 2 bears two composite heterozygous variants: c.1375C>T/c.1610C>T
184 (p.Arg459*/p.Thr537Ile). Thr537 is located in the PTP domain and is highly
185 conserved. Predictions for a substitution p.Thr537Ile, absent on GnomAD database,
186 were deleterious (SIFT, score 0.01) and probably damaging (PolyPhen-2, score
187 0.996).

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

191 In family 4, the homozygous stop variant c.1030C>T (p.Gln344*) was detected in
192 affected siblings in addition to a homozygous *MYO7A* gene mutation (c.5617C>T,
193 p.Arg1873Trp) related to the Usher syndrome type 1. A karyotype analysis was
194 performed in subject II₁ since *MYO7A* and *MTMR2* are in close chromosomal regions
195 (*MYO7A* at 11q13.5; *MTMR2* at 11q22). There was no deletion of this region. Both
196 parents carry the *MYO7A* variant and a heterozygous p.Gln344* *MTMR2* variant.

197

198 **3. Discussion**

199

200 In addition to the motor and sensory peripheral nerve involvement leading to severe
201 weakness and frequent loss of ambulation, the clinical spectrum of CMT4B1
202 encompasses cranial nerve involvement, claw hand and respiratory insufficiency.

203 Moreover, CMT4B1 is associated with a large genetic and phenotypic variability.

204 Although the phenotype was highly evocative in patient II₁ from family 1, CMT4B1
205 diagnosis was delayed and made after the diagnosis of Arnold-Chiari syndrome and
206 Usher syndrome, in families 2 and 4. In family 3 additional dysmorphic features, such
207 as macroglossia, large forehead and high-implanted hair have not been previously
208 described in association with CMT4B1.

209 Two cases of CMT1A associated with either an asymptomatic C2-C4[17] or a T11-L1
210 syringomyelia and urinary disorders[18] have been reported to date. Furthermore,
211 syringomyelia may be due to mutations in genes such as *GDF6* (8q22), *GDF3*
212 (12p13)[19] or *MEOX1* genes[20] related to Klippel-Feil syndrome. To our
213 knowledge, syringomyelia has not been reported in CMT4B1 patients.

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

217 Patient from family 3 presented incomplete common mesentery, a defect of
218 mesentery rotation rarely reported in adults. Recently, a case of CMT2S (*IGHMBP2*
219 mutation) associated with severe gastrointestinal dysautonomia was described[21].

220 To our knowledge, the only genetic syndromes manifesting with this intestinal
221 abnormality are Wolf-Hirschhorn and Pitt-Rogers-Danks syndromes, associated with

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

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

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

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

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

255 In family 2, a milder disease progression was observed in the three siblings carrying
256 a stop variant (p.Arg459*) combined with a missense variant p.Thr537Ile in the
257 phosphatase domain, potentially preserving a partial residual catalytic activity. There
258 are few CMTB1 milder cases reported[8,11]. Indeed, a patient with disease onset at
259 13 years still ambulant at 34 carried a homozygous frameshift variant in the extreme
260 C-terminus of *MTMR2* gene (p.Arg628fs*18)[13,11], between the CC and PDZ-BD
261 domains, affecting only PDZ-BD function, and preserving the domains responsible for
262 catalytic activity. Thus, a partially preserved function of MTMR2 protein could explain
263 milder phenotypes.

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

271

272 **Acknowledgements**

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

275

276

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279

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376

377 **Figure legends**

378

379 **Figure 1. A: Family pedigrees.** Black filled areas: affected individual; hatch symbol:
380 heterozygous individual; ?: unknown genetic, clinically asymptomatic; crossed white
381 symbol with black point: dead during infancy, epilepsy. **B-C: Spine MRI from Family**
382 **2, subjects I₁ (B) and II₁ (C):** syringomyelia. **D: Clinical findings in Family 4,**
383 **subject II₁:** claw hands with distal motor weakness and elbows contractures. **E-F:**
384 **Muscle biopsy findings.** Sural nerve biopsy from subject II₁ (Family 4). E: Semi-thin
385 section (thionin blue staining) shows a severe loss of myelinated fibers. Note the
386 presence of myelin outfoldings (arrows). F: Electronic microscopy revealed focally
387 folded myelin.

388

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

393

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

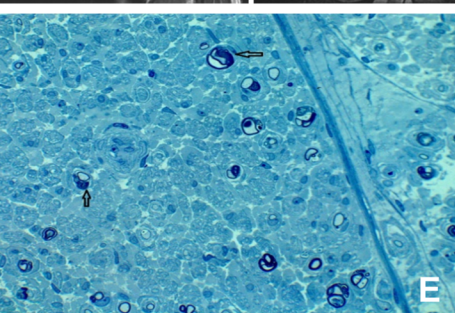
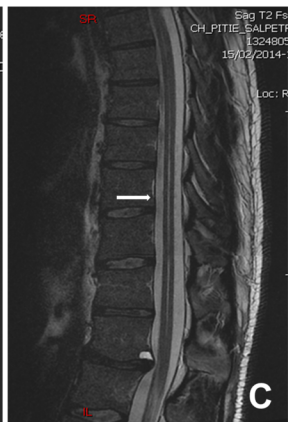
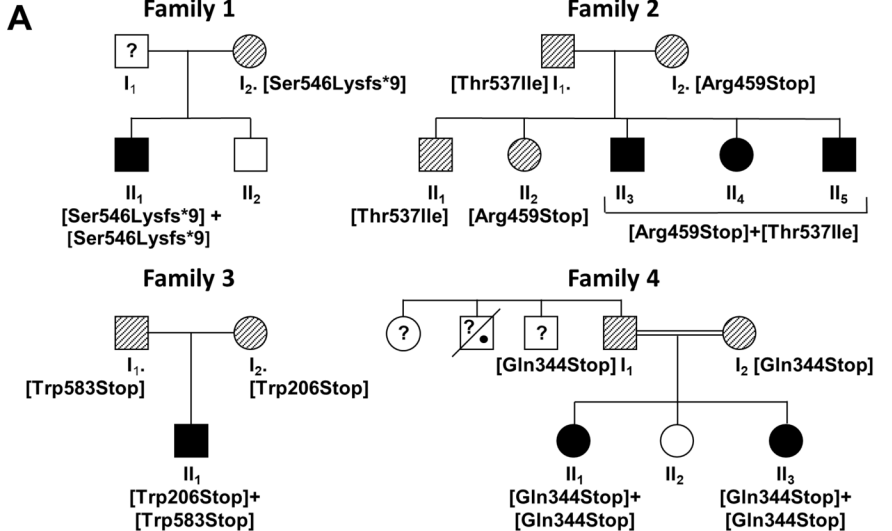
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Family	1		2			3	4	
Subject	II ₁	II ₃	II ₄	II ₅	II ₁	II ₁	II ₃	
cDNA change	[1633_1636dup] homozygous	[1375C>T]+ [1610C>T]			[617G>A]+ [1749G>A]	[1030C>T] homozygous		
Protein change	[Ser546Lysfs*9]	[Arg459*]+[Thr537Ile]			[Trp206*]+ [Trp583*]	[Gln344*]		
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	<i>Equinovarus</i>	<i>Cavus</i>	<i>Planus</i>	<i>Planus</i>	<i>Cavus</i>	<i>Cavus</i>	<i>Planus</i>	
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; NI: 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 (<i>tibialis anterior</i>)	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 (<i>abductor pollicis brevis</i>)	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 (<i>orbicularis oculi</i>)	Distal Latency (ms)	NO	NA	NA	NA	NA	6,5	≤3,1	
		Amplitude (mV)	NO	NA	NA	NA	NA	0,14	≥1	
	Hypoglossal (<i>genioglossus</i>)	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.