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1 **TITLE:** Leukoencephalopathy and conduction blocks in PLEKHG5-associated
2 intermediate CMT disease.

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30 **ABSTRACT**

31 Biallelic variants in *PLEKHG5* have been reported so far associated with different
32 clinical phenotypes including Lower motor neuron disease (LMND) [also known
33 as distal hereditary motor neuropathies (dHMN or HMN) or distal spinal muscular
34 atrophy (DSMA4)] and intermediate Charcot-Marie-Tooth disease (CMT). We
35 report four patients from two families presenting with intermediate CMT and
36 atypical clinical and para-clinical findings. Patients presented with predominant
37 distal weakness with none or mild sensory involvement and remain ambulant at
38 last examination (22-36 years). Nerve conduction studies revealed, in all patients,
39 intermediate motor nerve conduction velocities, reduced sensory amplitudes and
40 multiple conduction blocks in upper limbs, outside of typical nerve compression
41 sites. CK levels were strikingly elevated (1611-3867 U/L). CSF protein content
42 was mildly elevated in two patients. Diffuse bilateral white matter lesions were
43 detected in one patient. Genetic analysis revealed three novel frameshift variants
44 c.1835_1860del and c.2308del (family 1) and c.104del (family 2).

45 *PLEKHG5*-associated disease ranges from pure motor phenotypes with
46 predominantly proximal involvement to intermediate CMT with predominant distal
47 motor involvement and mild sensory symptoms. Leukoencephalopathy, elevated
48 CK levels and the presence of conduction blocks associated with intermediate
49 velocities in NCS are part of the phenotype and may arise suspicion of the
50 disease, thus avoiding misdiagnosis and unnecessary therapeutics in these
51 patients.

52

53 **KEY WORDS:** intermediate CMT; *PLEKHG5*; conduction block;
54 leukoencephalopathy; CSF protein; elevated CK

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57 **1. INTRODUCTION**

58 Hereditary motor and sensory neuropathies, classically known as Charcot-Marie-
59 Tooth disease (CMT), represent the commonest group of inherited
60 neuromuscular diseases [1]. These clinically and genetically heterogeneous
61 disorders are usually characterized by progressive distal muscle atrophy and
62 weakness, foot deformities and sensory loss.

63 Historically, based on electrophysiological findings, CMT were classified into
64 demyelinating forms (CMT1) when upper limb motor nerve conduction velocity
65 (MNCV) is significantly decreased (<38 m/s) and axonal forms (CMT2) with
66 normal or slightly reduced MNCV and significantly reduced amplitudes of motor
67 and sensory nerve action potentials. Additionally, intermediate CMT includes
68 forms with MNCV between those of typical CMT1 and CMT2 (usually between
69 35 and 45 m/s)[2]. Nerve biopsies in these intermediate forms show the
70 coexistence of axonal (degeneration of large axons and regenerative sprouting)
71 and demyelinating (thin or absent myelin sheaths, onion bulb formation)
72 abnormalities.

73 Recessive mutations in *Pleckstrin Homology And RhoGEF-domain-containing*
74 *G5* gene (*PLEKHG5*), initially reported in one consanguineous family with early-
75 onset severe lower motor neuron disease (or Distal Spinal Muscular Atrophy
76 [DSMA4]) [3], have been also formally associated with intermediate CMT [4,5].
77 More recently, cases with either pure lower motor neuron disease (LMND), distal
78 and proximal neuropathy with mild sensory involvement or intermediate-CM have
79 been documented [6–8]. Here, we report two additional families carrying novel

80 *PLEKHG5* mutations presenting with an intermediate CMT phenotype associated
81 with atypical findings, including leukoencephalopathy, conduction blocks on
82 electrophysiological studies and highly elevated creatine kinase (CK), thus
83 expanding the phenotypical spectrum of the disease.

84

85 **2. PATIENTS AND METHODS**

86 Four patients (3 women and 1 man) from two unrelated families were examined
87 by one of the authors. Nerve conduction studies and electromyography were
88 performed using standard techniques [9]. Definite conduction blocks were
89 defined by a drop in more than 50% in distal compound muscle action potential
90 (CMAP) amplitude, while probable conduction blocks were defined by a drop of
91 30-50% distal CMAP drop [10]. Brain MRI was performed in two patients while
92 muscle biopsy was conducted in one and processed for standard histological and
93 immunochemical studies [11].

94 DNA samples were extracted from peripheral blood samples and whole exome
95 sequencing (family 1) or next generation sequencing–based neuropathy
96 multigene panel (family 2) were performed. Sanger sequencing confirmed the
97 selected variants we identified. They were reported according to Human Genome
98 Variation Society recommendations (<http://varnomen.hgvs.org>).

99 GnomAD database (<http://gnomad.broadinstitute.org>) was used to search for the
100 variants' allele frequencies.

101 Informed consent was obtained from all patients, in agreement with local ethic
102 committees and with the 1964 Helsinki declaration and its later amendments

103

104 **3. RESULTS**

105 **3.1 Clinical findings**

106 Family 1

107 The two siblings from a non-consanguineous Vietnamese family (Figure 1A).
108 They have normal neuropsychological development. Patient III.5 presented with
109 distal lower limbs weakness and numbness first noted in adolescence with a
110 slowly progressive course. Examination revealed a steppage gait, mild distal
111 muscle atrophy, distal weakness (foot dorsiflexion 3/5), distal hypoesthesia in a
112 stocking-and-glove distribution and abolished deep tendon reflexes (DTRs). The
113 patient was able to walk without aid at last examination (Table 1). Patient III.4
114 had a more severe form with severe distal and proximal involvement of four limbs
115 (3/5 at distal and 4/5 at proximal UL, 2/5 distal 3/5 at proximal LL) but independent
116 ambulation was preserved. She had *pes cavus* and Achilles contractures.
117 Nerve conduction studies (NCS) disclosed reduced MNCVs in four limbs,
118 conduction blocks (>50%) in upper limbs (Figure 2A) affecting median and ulnar
119 nerves in the forearm out of typical nerve compression sites for patient III.4 while
120 for patient III.5 there was a definite conduction block affecting the median nerve
121 and a probable conduction block (-34.6%) affecting the ulnar. Most sensory nerve
122 action potentials (SNAPs) were abolished. Needle EMG of gastrocnemius muscle
123 from patient III.4 revealed giant MUAPs and poor recruitment indicating a chronic
124 neurogenic pattern but also the presence of complex repetitive discharges
125 (CRDs) (Figure 2C-D).
126 Interestingly, cerebrospinal fluid (CSF) analysis performed during the initial
127 diagnostic workup in patient III.5 revealed raised CSF protein level up to 0.70 g/L
128 (normal 0.15-0.45 g/L). Patient III.5 had also significantly raised serum creatine

129 kinase (CK) levels (1611 U/L). Cerebral MRI from the same patient was normal
130 aside from one millimetric white matter hyperintensity on T2-weighted images.

131

132 Family 2

133 The two siblings from family 2 were born to consanguineous marriage of parents
134 from Ivory Coast. Patient II.4 presented since early childhood with difficulty
135 running, poor sports performance and frequent falls. Subsequent progressive
136 distal upper and lower limb weakness was noted. Examination revealed
137 abolished DTRs in LL, predominantly distal weakness (UL 3/5, LL 4/5) but also
138 proximal UL (3/5) weakness. Distal reduced vibration sensation was noted with
139 no abnormalities on pinprick test. Her sister presented at age 20 with subacute
140 onset LL weakness. Clinical examination revealed *cavovarus* feet, hammer toes,
141 distal LL weakness (4/5) and mild proximal weakness (mainly hip extension
142 weakness), abolished DTRs and normal sensory examination. Both patients were
143 able to walk independently at last examination, but gait perimeter was limited for
144 patient II.4.

145 NCS from both siblings disclosed reduced MNCVs in four limbs as well as
146 moderately reduced SNAPs with intermediate sensory NCVs, especially for
147 patient II.4. Interestingly, probable conduction blocks in UL out of sites of potential
148 nerve compression (i.e. median nerve, Figure2B) were found for patient II.4 and
149 definite conduction blocks in the fibular nerve were noted in both cases. For
150 patient II.6, 20 and 24% drop in distal CMAP amplitude were detected in median
151 and ulnar nerves respectively, out of sites of potential nerve compression. Needle
152 EMG disclosed high-amplitude motor unit potentials (≥ 5 mV) with reduced
153 recruitment without spontaneous activity, suggesting a chronic neurogenic

154 process. Interestingly, CSF analysis of both patients revealed a slightly raised
155 protein content (0.68 g/L in patient II.5 and 0.53 g/L in II.6).
156 Similarly to patient III.5, patient II.4 had very raised CK levels (3867 U/L) (Table
157 1). Moreover, cerebral MRI disclosed posterior bilateral white matter lesions
158 (Figure 2E). Given the highly raised CK levels, a deltoid muscle biopsy was
159 performed, revealing fiber size variability, sparse necrotic fibers and mild fibrosis.
160 A genetic analysis of limb-girdle muscle dystrophies (LGMD)-causing genes by
161 next-generation sequencing (NGS), including *LAMA2*, was negative. A metabolic
162 workup (thyroid hormones, transferrin electrophoresis, phytanic acid,
163 arylsulfatase, galactocerebrosidase, cholestenol and long chain fatty acids
164 dosages and apolipoprotein profiles) conducted for patient II.4 was normal.

165 **3.2 Genetic findings**

166 Genetic analysis in the first family revealed two compound heterozygous variants
167 in *PLEKHG5*, NM_198681.3: i) the
168 c.1835_1860delGGCAGCGGCTGGCGGCCGTGGTGAGC (exon 16),
169 p.Arg612Profs*111, and affecting the Rho Guanine Exchange Factor (RhoGEF)
170 domain, and ii) the c.2308delG (exon 20), p.Glu770Serfs*72, and affecting the
171 Pleckstrin Homology (PH) domain (Figure 1B).

172 In family 2, the two siblings were homozygous for the c.104delG (exon2),
173 p.Cys35Phefs*100, while their mother was heterozygous.

174 These variants were absent in GnomAD (Table3).

175

176 **4. DISCUSSION**

177 Rapid progress in molecular genetics, including the increasing availability of next-
178 generation sequencing has allowed to identify over 100 genes associated to CMT

179 [1,12] and has unveiled a striking genetic and clinical overlap between CMT,
180 distal hereditary motor neuropathies (HMN) and lower motor neuron syndromes
181 [13,14]. Such is the case for *PLEKHG5*, associated with both proximal SMA,
182 intermediate CMT and HMN [3–6].

183 *PLEKHG5* is predominantly expressed in the central and peripheral nervous
184 system [15]. It has been shown to regulate autophagy of synaptic vesicles in axon
185 terminals of motoneurons, its depletion leading to impaired autophagy and
186 defective axon growth in cultured motoneurons [16]. Its RhoGEF domain involved
187 in the activation of NK-kB signaling pathway contributes to the activation of
188 GTPases which are in turn implicated in signaling mechanisms that regulate
189 neuronal plasticity, axonal growth, synapse formation, actin cytoskeleton
190 dynamics and neuronal survival[3,16–18]. The PH domain has been shown to
191 regulate the RhoGEF domain. *Plekhg5* inactivation in a murine model results in
192 a late-onset motoneuron disease with degeneration of axon terminals[16] but also
193 defective axon/Schwann cell units characterized by myelin infoldings in
194 peripheral nerves[19]. Moreover, aggregate formation have been observed in
195 mutant *Plekhg5* murine motoneurons[3]. Finally, sural nerve biopsies in
196 intermediate forms of *PLEKHG5*-related CMT have revealed severe loss of
197 myelinated fibres [4] while they showed no abnormalities in patients presenting
198 with the SMA form [3], suggesting different pathomechanisms might be involved.

199 So far, fifteen families carrying biallelic *PLEKHG5* pathogenic variants have been
200 reported [3–8]. Patients presented with either LMND (or DSMA4), in the first
201 family reported [3] and in more recently reported patients[6,7], or with a proximal
202 and distal motor neuropathy with initial prominent proximal weakness and mild
203 sensory involvement in 3 families [6], or with an intermediate CMT phenotype

204 exhibiting significant clinical and neurophysiological sensory involvement in six
205 families [4–6].

206 We report here four additional patients from two unrelated families presenting
207 with predominantly distal weakness and variable mild sensory involvement. Age
208 at onset was variable, ranging from early childhood in one patient to 15-20 years
209 in the three others. Neither spinal deformities were noted, nor cranial nerve,
210 respiratory nor cardiac involvement were detected. Neurophysiological studies
211 were compatible with an intermediate CMT but revealed in all four patients
212 multiple conduction blocks out of typical nerve compression sites. The latter
213 finding can sometimes hinder the diagnosis of a hereditary neuropathy, especially
214 in the absence of a known family history or when atypical clinical features are
215 present. Indeed, conduction blocks, raised CSF protein levels and the occurrence
216 of a proximal weakness may lead to discuss the diagnosis of chronic
217 inflammatory polyradiculoneuropathy leading to the prescription of intravenous
218 immunoglobulins, steroids or other immunosuppressive therapies. As such, this
219 have been described in different forms of CMT, either dominant, X-kinked or
220 recessive: i.e. CMT4J, caused by recessive mutations in the phosphoinositide
221 phosphatase *FIG4* gene CMT1B with *MPZ* mutations, CMT1C due to *LITAF*
222 mutations, CMTX due to *GJB1* mutations or CMT4C caused by recessive *SH3TC*
223 mutations [20–25].

224 CK levels in two of our patients were strikingly elevated as seen in two previously
225 reported patients[6], which may be part of the spectrum of *PLEKHG5*-related
226 neuropathies. Raised CK levels have also been reported in other CMT forms such
227 as those associated with *MPZ* or *PMP22* pathogenic variants, amongst others
228 [26–28]. Nonetheless, this may lead to the suspicion of a muscle disorder and

229 hence to a muscle biopsy. Interestingly, brain MRI revealed diffuse white matter
230 abnormalities in patient III.5, as reported in one patient by Chen and
231 colleagues[6]. A history of muscle weakness along with raised CK levels and
232 leukoencephalopathy should raise suspicion of a muscle dystrophy due to
233 *LAMA2* mutations [29,30] which have been excluded in our patient. Moreover,
234 white matter abnormalities have been classically described associated with
235 metabolic disorders such as lysosomal leukodystrophies (i.e. Krabbe or
236 metachromatic leukodystrophy) which may present with peripheral neuropathy
237 [31,32].

238 Central nervous system involvement and particularly diffuse white matter lesions
239 have also been reported in CMT patients with mutations in different genes, such
240 as *PMP22* [33], *GJB1* [34], *MFN2* [35] or *NEFL* [36]. Interestingly, a recent study
241 using brain diffusion tensor imaging (DTI) in a subset of 45 CMT patients has
242 shown abnormalities in 35 (including CMT1E/2A/2E/X1 forms) including
243 decreased fractional anisotropy and axial diffusivity with increased radial
244 diffusivity, suggesting white matter involvement albeit normal brain MRI imaging
245 in all patients [37].

246 *PLEKHG5* is also expressed in the central nervous system [15]. Interestingly, it
247 has been found to be involved in glioma tumor cell migration and invasion [38].
248 So far, diffuse brain white matter changes have been reported in one patient with
249 *PLEKHG5* mutations [6]. We report the second case here, suggesting that
250 leukoencephalopathy may indeed be part of the clinical spectrum, but further
251 reports and experimental data are needed to confirm this observation.

252 Genetic analysis revealed three previously unreported mutations in our patients.
253 All of them are of small frameshift deletions leading to premature termination
254 codons (PTC). The transcribed mRNAs from the two first fragment variants
255 (family 1: c.1835_1860del and c.2308delG) are predicted to be degraded by the
256 non-sense mRNA mediated decay (NMD) pathway since they lead to PTC
257 located more than 50–55 nucleotides upstream of exon–exon junction triggering
258 efficient NMD [39,40]. The c.104delG (p.Cys35Phefs*100) variant (exon2) is
259 located 39 nucleotides upstream of the 3' exon 5 junction complex and thus
260 predicted to escape the NMD machinery, and being either repressed by the
261 nonsense-mediated translational repression system (NMTR), as reported in the
262 literature [41], or leading to a truncated protein which is supposed to be potentially
263 eliminated by the Ubiquitin Proteasome Pathway. Experimental studies would
264 be needed in order to clarify this point.

265

266 Despite the different clinical phenotypes reported so far, no genotype-phenotype
267 correlations have been identified to date. Truncating as well as missense variants
268 affecting both main functional domains, inter-domains, N-terminal or C-terminal
269 parts of the protein, were associated with either CMT[4,5] or LMND (HMN,
270 DSMA4 or SMA)[3,7,8,38]. The site of the mutation and the potential involvement
271 of the functional protein domains or the predicted impact of the mutation have not
272 been associated with the phenotype variability among reported families. Intra-
273 familial variability in terms of severity, age at onset and course of the disease, as
274 seen in the two families reported here, has also been reported [4].

275 In conclusion, *PLEKHG5*-associated disease encompasses a continuum
276 phenotypical spectrum from LMND (DSMA, HMN) to intermediate CMT, including

277 also a range of motor-predominant hereditary neuropathies with proximal onset.
278 Our results expand the clinical and electrophysiological spectrum of the disease,
279 confirming diffuse white matter abnormalities and highly increased CK levels but
280 also revealing the presence of previously unreported conduction blocks on NCS.
281 The awareness and recognition of this features can contribute to a
282 better interpretation of novel *PLEKHG5* genetic variants. The appropriate
283 recognition of symptoms associated to an inherited neuropathy (motor signs
284 during childhood, foot deformities, scoliosis, predominant distal involvement) are
285 clue features to distinguish acquired neuropathies such as CIDP from
286 hereditary sensorimotor neuropathy with conduction block and thus to avoid
287 inappropriate therapeutic strategies, specially immunosuppressive treatments.

288

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- 446

447 **FIGURE LEGENDS**

448 **Table 1. Summarized clinical and ancillary tests findings.**

449 ^a (-) no weakness, (+) 4/5 on Medical Research Council (MRC) scale, (++) < 4/5
450 on MRC scale, (+++) complete paralysis; ^b(-) no atrophy, (+) mild atrophy, (++)
451 moderate atrophy, (+++) severe atrophy; ^c (-) normal, (+) mild hypoesthesia, (++)
452 profound hypoesthesia; ^d (+) normal, (+/-) decreased, (-) areflexia; CK= creatin
453 kinase; CSF=Cerebrospinal fluid; LL= Lower limbs; N= normal values; NA = no
454 data available; NCS= nerve conduction studies; UL= upper limbs; U/L= units/liter
455

456 **Table 2. Summarized EMG findings.** ^a Motor nerve conduction studies (MNCS).

457 CMAP: compound motor action potentials (mV). Muscles recorded: median:
458 abductor pollicis brevis (APB) / ulnar: adductor digiti minimi / superficial peroneal:
459 extensor digitorum brevis / tibial: tibialis posterior. AE: above-elbow. BE: below
460 elbow. BFH: below fibular head. PF: popliteal fossa. Normal CMAP values:
461 Median-Ulnar > 6; Peroneal > 3; Tibial > 6. Conduction blocks are indicated in
462 bold type. MNCV: motor nerve conduction velocity (m/s). Normal MNCV values:
463 Median-Ulnar > 42; Peroneal-Tibial 42. Distal latencies and F latencies are
464 indicated in ms. ^b Sensory nerve conduction studies (SNCS). SNAP: sensory
465 nerve action potential (μ V). Normal SNAP values: Median >15; Ulnar >8;
466 Superficial peroneal-Sural > 10. SNCV: Sensory conduction velocities (m/s;
467 orthodromic). SNCV normal values= Median-Ulnar >45; Superficial peroneal-
468 Sural 40.

469 NA= not ascertained. NR = not recordable

470

471

472 **Table 3. Summarized information of the reported novel variants.** Het:
473 heterozygous, Hom: homozygous; NGS: next generation sequencing; WES:
474 whole exome sequencing

475

476 **Figure 1. (A) Family pedigrees.** Black-filled symbols: affected individuals. Black-
477 arrows: index cases. Electropherograms indicating the corresponding genetic
478 variants. **(B) Schematic representation of the *PLEKHG5* genomic sequence**
479 **(upper panel) and protein (lower panel), including the main functional**
480 **domains, based on NCBI reference sequence NM_198681.3.** Localization of
481 the novel mutations reported here (red) and the previously reported mutations
482 (black). PH : Pleckstrin Homology domain. RBD : Ras Binding Domain.
483 RhoGEF : Rho Guanine Exchange Factor domain.

484

485 **Figure 2. Neurophysiological and imaging findings.** **(A)** Conduction block on
486 ulnar motor NCS recorded on *abductor digiti minimi* in patient III.5 from family 1.
487 **(B)** Multiple conduction blocs on UL in patient II.4 from family 2. Conduction block
488 on median nerve (recorded on *abductor pollicis brevis*) **(A)** and ulnar nerves
489 (recorded on *abductor digiti minimi*) **(B)**. Note reduced CMAP amplitude on
490 proximal (elbow) stimulation compared with distal (wrist) stimulation. **(C-D)**
491 Needle EMG of gastrocnemius muscle from patient III.4 from family 1 showing
492 complex repetitive discharges **(C)** along with giant MUAPs and poor recruitment
493 indicating a chronic neurogenic pattern. **(E)**: Brain MRI from patient 2-II.4. T2
494 FLAIR-weighted axial images reveal diffuse and bilateral periventricular white
495 matter lesions (white arrows). **(F-G)**: Note distal wasting, *cavus* feet and hammer
496 toes (patient 2-II.4). **(H)**: Muscle MRI (lower limbs) from patient 2-II.4 (axial, T1-

497 weighted) reveals mild fatty degeneration affecting quadriceps and sartorius in
498 thighs. Note prominent atrophy and fatty degeneration of gastrocnemius and
499 soleus muscles.

500