Leukoencephalopathy and conduction blocks in PLEKHG5-associated intermediate CMT disease

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ABSTRACT

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

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

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

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1. INTRODUCTION

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

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

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

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

2. PATIENTS AND METHODS

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

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

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

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

3. RESULTS

3.1 Clinical findings

Family 1

The two siblings from a non-consanguineous Vietnamese family (Figure 1A). They have normal neuropsychological development. Patient III.5 presented with distal lower limbs weakness and numbness first noted in adolescence with a slowly progressive course. Examination revealed a steppage gait, mild distal muscle atrophy, distal weakness (foot dorsiflexion 3/5), distal hypoesthesia in a stocking-and-glove distribution and abolished deep tendon reflexes (DTRs). The patient was able to walk without aid at last examination (Table 1). Patient III.4 had a more severe form with severe distal and proximal involvement of four limbs (3/5 at distal and 4/5 at proximal UL, 2/5 distal 3/5 at proximal LL) but independent ambulation was preserved. She had *pes cavus* and Achilles contractures.

Nerve conduction studies (NCS) disclosed reduced MNCVs in four limbs, conduction blocks (>50%) in upper limbs (Figure 2A) affecting median and ulnar nerves in the forearm out of typical nerve compression sites for patient III.4 while for patient III.5 there was a definite conduction block affecting the median nerve and a probable conduction block (-34.6%) affecting the ulnar. Most sensory nerve action potentials (SNAPs) were abolished. Needle EMG of gastrocnemius muscle from patient III.4 revealed giant MUAPs and poor recruitment indicating a chronic neurogenic pattern but also the presence of complex repetitive discharges (CRDs) (Figure 2C-D).

Interestingly, cerebrospinal fluid (CSF) analysis performed during the initial diagnostic workup in patient III.5 revealed raised CSF protein level up to 0.70 g/L (normal 0.15-0.45 g/L). Patient III.5 had also significantly raised serum creatine

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

Family 2

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

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

process. Interestingly, CSF analysis of both patients revealed a slightly raised protein content (0.68 g/L in patient II.5 and 0.53 g/L in II.6).

Similarly to patient III.5, patient II.4 had very raised CK levels (3867 U/L) (Table 1). Moreover, cerebral MRI disclosed posterior bilateral white matter lesions (Figure 2E). Given the highly raised CK levels, a deltoid muscle biopsy was performed, revealing fiber size variability, sparse necrotic fibers and mild fibrosis. A genetic analysis of limb-girdle muscle dystrophies (LGMD)-causing genes by next-generation sequencing (NGS), including *LAMA2*, was negative. A metabolic workup (thyroid hormones, transferrin electrophoresis, phytanic acid, arylsulfatase, galactocerebrosidase, cholestenol and long chain fatty acids dosages and apolipoprotein profiles) conducted for patient II.4 was normal.

3.2 Genetic findings

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

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

These variants were absent in GnomAD (Table3).

4. DISCUSSION

Rapid progress in molecular genetics, including the increasing availability of next-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

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

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

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

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

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

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

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

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

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

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

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FIGURE LEGENDS

Table 1. Summarized clinical and ancillary tests findings.

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

Table 2. Summarized EMG findings. [a] Motor nerve conduction studies (MNCS). CMAP: compound motor action potentials (mV). Muscles recorded: median: abductor pollicis brevis (APB) / ulnar: adductor digiti minimi / superficial peroneal: extensor digitorum brevis / tibial: tibialis posterior. AE: above-elbow. BE: below elbow. BFH: below fibular head. PF: popliteal fossa. Normal CMAP values: Median-Ulnar > 6; Peroneal > 3; Tibial > 6. Conduction blocks are indicated in bold type. MNCV: motor nerve conduction velocity (m/s). Normal MNCV values: Median-Ulnar > 42; Peroneal-Tibial 42. Distal latencies and F latencies are indicated in ms. [b] Sensory nerve conduction studies (SNCS). SNAP: sensory nerve action potential (μV). Normal SNAP values: Median >15; Ulnar >8; Superficial peroneal-Sural > 10. SNCV: Sensory conduction velocities (m/s; orthodromic). SNCV normal values= Median-Ulnar >45; Superficial peroneal-Sural 40.

NA= not ascertained. NR = not recordable

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

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

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