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CASE STUDY

Novel *PLEKHG5* mutations in a patient with childhood-onset lower motor neuron disease

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Abstract

The *PLEKHG5* gene encodes a protein that activates the nuclear factor kappa B (NFκB) signaling pathway. Mutations in this gene have been associated with distal spinal muscular atrophy IV and intermediate axonal neuropathy C, both with an autosomal recessive mode of inheritance. Two families with low motor neuron disease (LMND) caused by mutations in *PLEKHG5* have been reported to date. We present a third LMND family, the first nonconsanguineous, due to two not previously reported *PLEKHG5* mutations. Our results confirm and extend previous findings linking *PLEKHG5* mutations to lower motor neuron diseases.

Background

PLEKHG5 (pleckstrin homology and RhoGEF domain containing G5), codes for a guanine exchange factor (GEF) specifically expressed in motor neurons, where it regulates autophagy of synaptic vesicles. Furthermore, PLEKHG5 plays a role in the activation and regulation of RhoA signaling pathways, thereby controlling neuronal cell differentiation and maintaining stable cell-cell contacts. In addition, PLEKHG5 is required for the polarity of actively migrating brain cells, but also breast tumor cells.[1–3] Mutations in *PLEKHG5* appear to be involved in cell death through impaired NF-κB signaling,[4,5] and are responsible for different forms of motor neuron diseases such as distal spinal muscular atrophy IV (DSMA4; MIM 611067) and intermediate axonal neuropathy C (CMTRIC; MIM 615376), both of which have an autosomal recessive pattern of inheritance.[4,6–10]

Inherited lower motor neuron diseases (LMNDs) encompass a genetically heterogeneous group of clinical disorders affecting the distal motor nerve up to the level of the anterior horn cell. They are characterized by progressive muscle weakness (distal or proximal) with atrophy and hypo/areflexia, without sensory involvement. Classification of these syndromes is complex and not standardized, but a variety of hereditary causes is recognized. Differential diagnosis between LMNDs is difficult because symptoms often overlap and phenotypes and genotypes vary widely. With the development of next-generation sequencing (NGS) the number of genetic

causes of LMNDs is expanding, and novel genetic variants are being identified. Several causative genes have been reported, with varying clinical manifestations, mode of inheritance, and age of onset.[11–13]

To date, LMND caused by mutations in *PLEKHG5* has been reported in two families.[4,8,9] Here we present the first nonconsanguineous LMND case due to two *PLEKHG5* frameshift mutations not previously described.

Case Presentation

A 33-year-old Spanish male presented with a history of progressive and symmetric proximal and distal limb weakness, predominantly in the legs, since age 8, and loss of ambulation at age 17. He was an only child and the family had no history of consanguinity. Physical examination showed scapula winging and the absence of deep reflexes. Facial involvement and upper motor neuron signs were absent. Hyperlordosis and contractures of hips and elbows were also noted. No respiratory or bulbar impairment was observed.

Lab tests revealed mild hyperCKemia (two to threefold above the normal range). Muscle Computerized Tomography (CT) showed a predominant involvement of glutei and anterior muscle group at thighs (Figure 1).

Nerve conduction studies were normal and the needle EMG showed denervation with the presence of fibrillation potentials, motor unit action potentials (MUAPs) with increased duration and amplitude, and a reduced recruitment pattern. The muscle biopsy was consistent with a denervation pattern. A clinical diagnosis of lower motor neuron disease was established.

Methods

DNA was extracted from peripheral blood following standard procedures. The *SMN1* gene was analyzed by MPLA (SMA Salsa Kit P021, MRC Holland, Netherlands) and sequencing of the whole-coding region. We sequenced the proband's DNA using a custom-targeted NGS gene panel including 116 neuromuscular disease-related genes,

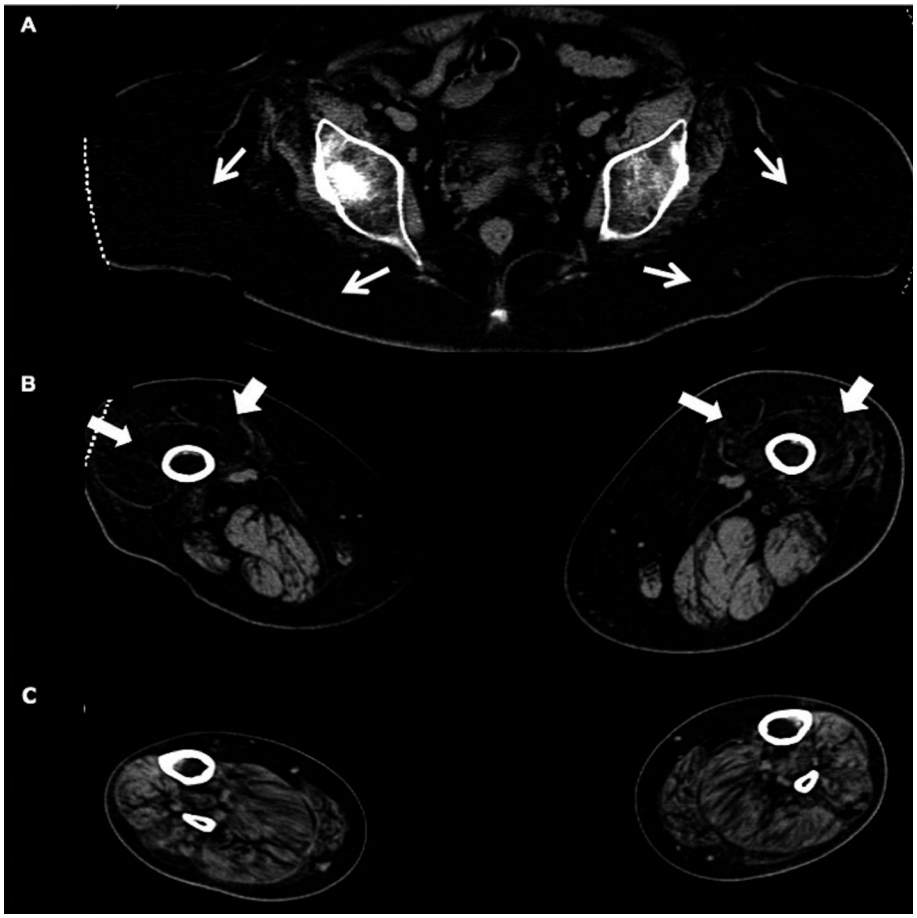

Figure 1. Axial computed tomography (CT) images of the pelvis (A), thigh (B), and lower leg (C) of the studied patient. Note the different degrees of fatty degeneration (with low density). The most affected muscles are the glutei (*thin white arrow* in A), and anterior muscle group (*thick white arrow* in B). In contrast, muscles of the lower legs are comparatively preserved (C).

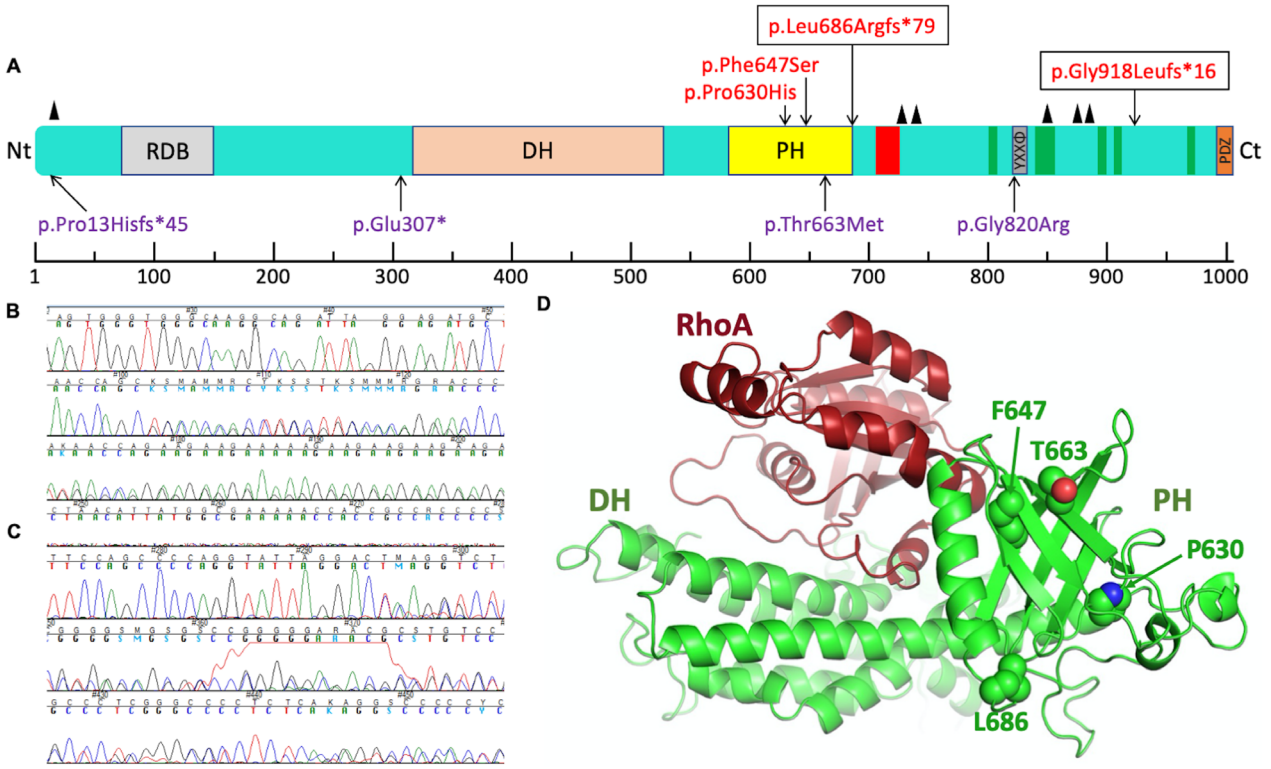

Figure 2. Schematic representation of the domain organization in PLEKHG5 (A), showing the position of mutations identified in our patient (boxed) and in previous reports. Mutations described in LMND and iCMT patients are indicated in red and purple, respectively. RDB: Rnd3-binding domain; DH: Dbl homology domain; PH: Pleckstrin homology domain; PDZ: PDZ binding motif; YXXΦ: tyrosine-based sorting motif; Nt: N-terminus; Ct: Ct-terminus. The red box indicates an extremely acidic Glu-rich stretch in the wild-type protein, which is replaced by a highly basic sequence in the p.Leu686Argfs*79 mutant. Green boxes highlight putative SH3-binding motifs. Ser/Thr phosphorylation sites are marked with triangles. Sequence electropherograms showing the patient mutations: c.2057delT in heterozygosis in exon 19 of *PLEKHG5* (B); c.2752_2753delGG in heterozygosis in exon 20 of *PLEKHG5* (C). Model of the PLEKHG5-RhoA complex showing the reported mutants in the PH domain (D). We used a numbering system based on PLEKHG5 isoform a. For the numbering system favored in protein databases, add +56.

following Nextera Rapid Capture technology (Illumina, San Diego, CA). We used 50 ng of genomic DNA to construct the library following the manufacturer's protocols. Sequencing was performed using the MiSeq Platform (Illumina). Variant annotation and filtering were undertaken using Illumina Variant Studio 3.0 (Illumina), starting with variant call format (VCF) files from each sample. Selected variants had a read depth above 30× and a variant frequency reported in Thousand Genomes, Exome Aggregation Consortium (ExAC), Genome Aggregation Database (gnomAD), and Exome Variant Server (EVS) below 5%. Variant pathogenicity was assessed according to the American College of Medical Genetics (ACMG) guidelines.[14] Clinically relevant variants were confirmed by Sanger sequencing and segregated in the family. *PLEKHG5* mutations were named based on RefSeq NM_020631.4 in agreement with previous reports.

Written informed consent was obtained from the patient and his parents. The genetic study was approved by the Ethics Committee at Hospital de la Santa Creu i Sant Pau (HSCSP) and was performed in accordance with the ethical principles of the Helsinki Declaration (WMA, 2013).

Results

SMN1 gene analysis did not reveal any disease-causative mutation. However, the custom-targeted NGS gene panel identified two novel compound heterozygous frameshift mutations in *PLEKHG5* as the best candidate variants responsible for the clinical findings: 1) c.2057del (p.Leu686Argfs*79) located in exon 19; and 2) c.2752_2753del (p. Gly918Leufs*16), in exon 20 (Figure 2). This finding was corroborated by the patient's participation in the Navarra 1000 Genomes project (NAGEN 1000). Segregation analysis concluded that c.2057del was inherited from the paternal allele, whereas c.2752_2753del was inherited from the maternal allele, confirming their recessive inheritance.

Table 1. Summary of the reported *PLEKHG5* reported cases

Family	*PLEKHG5* mutations*	Phenotype/ Age at onset	Clinical findings[a]	Structural and functional impact
1	p.Phe647Ser p.Phe647Ser	Severe LMND/ 2 to 3.5 years	Proximal and distal muscle weakness predominating at the lower limbs with generalized areflexia. Contractures, severe scoliosis, and hyperlordosis. Loss of ambulation at the mean age of 8.5 years. Assisted ventilation is needed at a mean age of 16.5 years. Muscle denervation and slightly decreased motor nerve conduction velocities.	Unfolding of PH domain, likely followed by protein degradation.
		Mild LMND/ 11.5 years	Moderate generalized muscle weakness and slow course of the disease. Hyperlordosis and bilateral winging of scapula. All deep tendon reflexes were abolished. Muscle denervation and subnormal motor and sensory nerve conduction velocities.	
2	p.Thr663Met p.Gly820Arg	CMTRIC/ 8 years	Muscle weakness and atrophy of bilateral distal muscles, predominantly at lower limbs. Bilateral pes cavus and stepage gait. Decreased sensitivity. Decreased motor and sensory nerve conduction velocities.	No major structural impact. Might disrupt binding site for putative macromolecular partner. Loss of binding sites for regulatory factors (e.g., PDZ and SH3-containing proteins).
3	p.Pro13Hisfs*45 p.Pro13Hisfs*45	CMTRIC/ 7 years and 20 years	Limb-girdle muscle weakness and atrophy, foot deformities, moderately decreased nerve conduction velocities.	No functional protein was produced.
4	p.Glu307* p.Glu307*	CMTRIC/ Between the second and the fifth decade.	Limb-girdle muscle weakness and atrophy, foot deformities, moderately decreased nerve conduction velocities, loss of large myelinated fibers, and thin myelination	Truncated protein produced that lacks major functional domains (DH/ PH); likely degraded.
5	p.Pro630His p.Pro630His	LMND/ 13 years	Lower motor neuron type of disease with slow progression	Unfolding of PH domain, likely followed by protein degradation.
6	p.Leu686Argfs*79 p.Gly918Leufs*16	LMND/ 8 years	Progressive and symmetric proximal and distal limb weakness, predominantly in the legs. Loss of ambulation at age 17. Scapula winging and absence of deep reflexes. Hyperlordosis and contractures of hips and elbows. Mild hiperCKemia. Denervation pattern with normal nerve conduction studies.	Loss of binding sites for regulatory factors (e.g., PDZ and SH3-containing proteins). In p.Leu686Argfs*79, additional loss of acidic stretch and of several phosphorylation sites.

[a]Clinical and molecular findings have been compiled from the following references: Family 1: Maystadt I, et al. Neurology 2006 and Maystadt I et al. AJHG 2007. Family 2: Kim HJ, et al. Orphanet J. Rare Dis. 2013. Family 3 and 4: Azzedine H, et al. Hum. Mol. Genet. 2013. Family 5: Özoğuz A, et al. Neurobiol. Aging 2015. Family 6: the present work.

Discussion

Six different *PLEKHG5* mutations have been described in five different families to date, two of which presented LMND,[4,9] whereas the other three presented intermediate Charcot-Marie-Tooth (iCMT) forms[7,10] (Figure 2A and Table 1). Here we describe a third family with childhood-onset and autosomal recessive inheritance of LMND, which is apparently caused by two novel compound heterozygous frameshift mutations in *PLEKHG5*.

Mutation p.Leu686Argfs*79 affects a residue that marks the C-terminal end of the PH domain and the start of a long region predicted to be intrinsically disordered in solution. Even though the aliphatic side chain of Leu686 might engage in Van-der-Waals interactions with other residues of this domain (Figure 2D), its nonconservative exchange by an arginine is unlikely to have a significant structural impact. Replacement of the C-terminal region by a fully unrelated peptide seems to have much more important functional consequences. Most notably, there is a reversal-of-charge in the downstream Glu705-Asp726 stretch of the protein (Figure 2A), from extremely acidic (mostly glutamate residues) to highly basic (mostly arginines). This polarity change is likely to result in impaired interactions of PLEKHG5 with phospholipid membranes. This is in addition to the loss of several additional protein–protein binding sites such as the tyrosine-based sorting

motif, Tyr819-Xxx-Xxx-Leu822, responsible for the interaction with the μ-subunit of Adaptor Protein complex as well as Ser/Thr phosphosites at positions 729, 734, 851, 876, and 881.

Mutation p.Gly918Leufs*16 replaces the last C-terminal 89 residues of the protein by an unrelated, shorter 16-residue peptide. Both mutants lack the C-terminal PDZ-binding motif, Leu1001-Val1006, conserved in all isoforms of the protein but isoform 4. These truncated PLEKHG5 variants also lack several proline-rich stretches that are binding motifs for SH3-containing proteins, most notably the Arg967-Pro973 heptapeptide, which could interact with both class I and class III SH3 domains. Altogether, the C-terminal region of this GEF appears to be involved in critically important interactions with other macromolecules, including perhaps specific membrane domains, and impaired binding to these interactors would explain the observed phenotype. PLEKGH5 regulates autophagy of synaptic vesicles in the axon terminal of motor neurons, leading to an accumulation of synaptic proteins and resulting in dysfunction of neuromuscular junctions and motoneurons.[15] Conceivable, the two mutations identified in the current work impair protein–protein and/or protein-membrane interactions needed during the complex autophagy process. Since these data come from *in silico* predictions, it would be desirable to perform functional tests to analyze the stability of the protein and the functional impact of the identified mutations. Unfortunately, we do not have a tissue sample from the patient to perform these analyses.

By contrast, the causative mutations in other LMND families previously reported, p.Pro630His and p.Phe647-Ser, do affect the core of the PH domain and would profoundly impair its folding[4,9] (Figure 2D). Also along these lines, missense mutations in the PH domain of *PLEKHG5* (p.Thr663Met) and in the C-terminal region (p.Gly820Arg) have also been linked to autosomal-recessive iCMT disease.[7] Thr663 is relatively exposed to bulk solvent, and the mutation is unlikely to cause major structural rearrangements in the PH domain. However, its proximity to Phe647 might point to the disruption of a binding site for a PLEKHG5 cofactor, perhaps Rab26 itself (Figure 2D). On the other hand, mutant p.Gly820Arg maps to the Tyr-based sorting motif, Y819-L822. However, Arg820 might actually be preferred at the second position of this motif, as the basic residue is repeatedly found in internalized proteins such as the EGF receptor. These patients expressed low levels of PLEKHG5 in the distal sural nerve and *in vitro* studies suggested defects in the activation of the NF-kB signaling pathway as likely explanation for the deleterious effect of the identified *PLEKHG5* mutants.

Further studies are needed to better understand the pathogenic mechanisms of *PLEKHG5* and the different clinical manifestations of its mutations (e.g., LMND in some patients and iCMT in others). Interestingly, other PH domain-containing proteins have also been linked to neurodegenerative diseases in humans. For instance, mutations in the C-terminal half of dynamin 2 (*DNM2*) PH domain are associated with a dominant myopathic phenotype of centronuclear myopathy [MIM 160150], whereas mutations in the N-terminal half of the same domain cause dominant type B iCMT.[16]

The high-sequencing output of NGS techniques has allowed the identification of new disease-causing mutations thus furthering our understanding of the genetic basis of LMNDs. However, many LMND patients do not yet have a conclusive genetic diagnosis. The detailed clinical and molecular analysis of these cases can contribute to establish genotype–phenotype correlations, adding to our understanding of the pathogenic pathways underlying this heterogenous group of diseases.

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Authors' Contributions

LGQ contributed to the interpretation of the findings, wrote, and revised the draft of the manuscript. PFP performed the structural analyses, revised, and edited the manuscript. IP was involved in the acquisition and interpretation of imaging data and drafting and revising the manuscript. MJR and JSG performed the genetic analysis. SB contributed to the analysis and interpretation of the genetic findings. LT performed a bibliographic search. PG contributed to the interpretation of the findings and revised and edited the manuscript. IJ examined the patient and collected all the clinical data, wrote, and revised the draft of the manuscript.

Conflict of interest

All authors declare that they have no conflict of interest.

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