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Novel variants broaden the phenotypic spectrum of *PLEKHG5*-associated neuropathies

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Abstract

Background: Pathogenic variants in *PLEKHG5* have been reported, to date, to be causative in three unrelated families with autosomal recessive intermediate Charcot-Marie-Tooth disease (CMT) and in one consanguineous family with spinal muscular atrophy (SMA). *PLEKHG5* is known to be expressed in the human peripheral nervous system and previous studies have shown its function in axon terminal autophagy of synaptic vesicles lending support to its underlying pathogenetic mechanism. Despite this, there is limited knowledge of the clinical and genetic spectrum of disease.

Methods: We leverage the diagnostic utility of exome and genome sequencing and describe novel biallelic variants in *PLEKHG5* in thirteen individuals from nine unrelated families originating from four different countries. We compare our phenotypic and genotypic findings with a comprehensive review of cases previously described in the literature.

Results: We found that patients presented with variable disease severity at different ages of onset (8 to 25 years). In our cases, weakness usually started proximally, progressing distally, and can be associated with intermediate slow conduction velocities and minor clinical sensory involvement. We report three novel nonsense and four novel missense pathogenic variants associated with these *PLEKHG5*-associated neuropathies which are phenotypically SMA or intermediate CMT.

Conclusions: Therefore, *PLEKHG5*-associated neuropathies should be considered as an important differential in non-5q SMAs even in the presence of mild sensory impairment and a candidate causative gene for a wide range of hereditary neuropathies. We present this series of cases to further the understanding of the phenotypic and molecular spectrum of *PLEKHG5*-associated diseases.

ABSTRACT WORD COUNT = 247

Graphical/ brief abstract

We describe novel biallelic variants in *PLEKHG5* in thirteen individuals from nine unrelated families extending the phenotypic and molecular spectrum of *PLEKHG5*-associated disease. *PLEKHG5* pathogenic variants should be considered in motor-predominant hereditary neuropathies, usually starting proximally and can be associated with minor sensory involvement.

Background

Biallelic pathogenic variants in pleckstrin homology and RhoGEF-domain-containing G5 (*PLEKHG5*) have been linked to one family with distal spinal muscular atrophy type 4 (DSMA4) (MIM 611067)[1] and intermediate Charcot-Marie-Tooth disease (CMT) (MIM 615376) in three unrelated families[2,3]. Immunohistochemical analysis in the sural nerve biopsy of a CMT patient revealed low PLEKHG5 levels[3]. In spinal muscular atrophy (SMA), a homozygous mutation in the pleckstrin homology (PH) domain affected the nuclear factor-κB (NF-κB) transduction pathway[1]. Impaired synaptic vesicle autophagy is seen in PLEKHG5-depleted cultured motor neurons that show defective axon growth[4].

In this study, we report seven distinct variants in thirteen individuals from nine unrelated families presenting between 8 and 25 years of age. Most patients presented with proximal weakness, sometimes associated with intermediate slow conduction velocities and minor clinical sensory involvement in some individuals. These cases extend the spectrum of *PLEKHG5*-associated diseases, and when compared to those previously reported in the literature (**Figure 1A, Table 1**), further characterise the clinical heterogeneity of *PLEKHG5*-associated neuropathies.

Methods

All participants provided informed written consent for participation. We provide detailed clinical description of patients presenting to tertiary neurology units in Iran, Turkey, Germany and India. All patients underwent examination by a neurologist in peripheral nerve disease and neurophysiological assessment by a local specialist. Where indicated, further examination with brain and limb MRI as well as muscle biopsy (neuropathologist-reported) are presented. For genetic analyses, DNA was extracted from peripheral blood with consent. Some individuals underwent *SMN1* and *SMN2* testing through multiplex ligation-dependent probe amplification (MLPA) or quantitative molecular analysis using real-time PCR under consensus criteria[5]. Other individuals had targeted neuropathy panel sequencing (*SMN1, LMNA, MFN2, MPZ, GJB1, PMP22, SH3TC2, GDAP1, NEFL, DNAJB2, HINT*). Exome sequencing and interpretation was carried out as described previously[6-8]. Candidate variants were confirmed by Sanger sequencing. *In silico* predictions of pathogenicity were carried out using Sorting Intolerant from Tolerant (SIFT)[9], Prediction of Functional Effect of Human nsSNPs (PolyPhen)[10], Combined Annotation Dependent Depletion (CADD) scores[11] (**Table 2**), and conservation information from five species. Allele frequencies were interrogated through Genome Aggregation Database (gnomAD) v.21[12]; Iranome: a catalogue of variants from whole exome sequencing of 800 Iranian individuals[13]; Varbank Platform (1,657 sets of

exomes, Cologne Centre for Genomics); RD-Connect GPAP Platform (3,549 individual exomes) and the UCL Queen Square Institute of Neurology (QS IoN) inhouse database comprising exome sequencing data of 15,000 individuals.

Results

Family 1: Case 1

Patient 1 is a 34-year-old, right-handed Persian man, born to healthy consanguineous parents (first cousins) who had three other healthy children (**Figure 1B**). He presented with difficulty lifting both arms with cramps aged 19 years, followed by difficulty climbing stairs. Six years after initial symptom onset, both feet dragged on walking. At 31 years, he experienced numbness and paraesthesia affecting his hands and more severe numbness in his feet. He had an otherwise normal antecedent history. There was no family history of note. Examination revealed a symmetrical proximal limb weakness with milder distal weakness. There was left forearm and arm wasting (**Figure 2A**) with bilateral scapular winging (**Figure 2B**) and absent tendon reflexes. There was loss of light-touch, pinprick and vibration sensation to the medial malleoli and preserved arm sensation. There were no spine, hand or foot deformities. Bedside cognitive and cranial nerve examinations were normal. He had a waddling and high-stepping gait. Nerve conduction studies (NCS) aged 34 years (**Supplementary Table 1**) showed a predominant motor neuropathy with slowed motor nerve conduction velocities (MNCV) and normal compound muscle action potentials (CMAP) in the arms and legs. Sensory nerve conduction velocity (SNCV) was moderately slowed but sensory nerve action potentials (SNAP) were preserved. Electromyography (EMG) showed a chronic neurogenic process with some ongoing active denervation in proximal and distal, arm and leg muscles. Serum creatine kinase (CK) was 367 U/L (normal < 195). Leg muscle MRI showed proximal and distal involvement with moderate fatty infiltration of the medial head of gastrocnemius and vastus lateralis, and more mildly in the gluteus muscles (**Figure 2C**). Brain imaging showed diffuse increased signal in the deep white matter (**Figure 2D**). Taken together, the clinical and neurophysiology results are consistent with a proximal and distal neuropathy with intermediately slowed conduction velocities and minor sensory involvement clinically.

Quantitative PCR found that the patient carried a heterozygous *SMN1* deletion but further targeted sequencing showed no pathogenic variant on the other allele. Exome sequencing revealed a homozygous frameshift variant in *PLEKHG5* [c.79_83del: NM_198681.3 (p.Pro27Ter)]. Segregation analysis by Sanger sequencing showed that the variant co-segregates with disease (**Figure 1B**). This variant is present in one

of 227,304 alleles in gnomAD (minor allele frequency (MAF) = 4.4E-6)[12] and has not been described in Iranome[13] or QS IoN database (**Table 2)**.

Family 2: Case 2

Presenting to the same unit was a 19-year-old, right-handed Persian female who complained of lower limb weakness since age 8 years. She was born to healthy consanguineous parents who were first cousins and had an asymptomatic 12-year-old sister (**Figure 1C**). There is no family history of note. She initially noticed difficulty climbing stairs. Two years after onset, weakness progressed to both arms. She then experienced a weakened grip and bilateral foot drop. This culminated in her being wheelchair-bound over the last decade. Cranial nerve examination was normal. Bilateral scapular winging was present. Limb weakness affected proximal more than distal muscle groups: there were no antigravity movements in the shoulders or elbows and only a trace of movement at the hips and knees. She had mild weakness in the hands (left more than right) (**Figure 2E**) and mild ankle weakness. She had prominent lumbar hyperlordosis (**Figure 2F, G**) without obvious wasting or contractures. Tendon reflexes were absent. There was no evidence of any sensory deficit. Motor and sensory NCS were normal at 8 years (**Supplementary Table 1**). EMG showed giant motor units with reduced recruitment and no evidence of active denervation, predominantly at proximal sites, consistent with SMA. A repeat EMG aged 19 years showed evidence of more proximal than distal chronic neurogenic process. NCS showed preserved CMAPs but a decrement from those observed ten years previously. CK was normal. Muscle MRI showed severe muscle atrophy and fatty replacement more in the thighs (**Figure 2H**) than the legs (**Figure 2I**) reflecting more proximal involvement. MRI brain was normal.

MLPA in *SMN1* showed no abnormalities. Exome sequencing revealed the same homozygous frameshift variant as Case 1 in *PLEKHG5* [c.79_83del: NM_198681.3 (p.Pro27Ter)]. Segregation analysis by Sanger sequencing showed her healthy parents are heterozygous carriers.

Family 3: Case 3

The third patient is a 44-year-old Turkish lady born of consanguineous parents. She is the youngest of seven asymptomatic siblings. Her 77-year-old father is well. Her mother died aged 68 from stroke. She presented aged 14 years with proximal lower limb weakness which gradually progressed to involve the distal arms and legs. Examination revealed a picture of pure lower motor neuron involvement in the

proximal and distal lower and distal upper limbs (**Supplementary Video 1**). There was no upper limb wasting **(Figure 2J, K**). Bedside cognitive examination was normal. NCS showed slow MNCVs and reduced CMAPs with no sensory involvement (**Supplementary Table 1**). EMG showed chronic neurogenic changes and muscle biopsy confirmed neurogenic changes. Analysis of *SMN1* revealed no abnormalities. Exome sequencing revealed the same homozygous variant in *PLEKHG5* [c.79_83del: NM_198681.3 (p.Pro27Ter)] as Cases 1 and 2. Her father, one brother and one sister were found to be carriers by Sanger sequencing (**Figure 1D**).

Family 4: Cases 4, 5 and 6

Presenting to the same unit in Turkey were three affected siblings born of consanguineous parents (first cousins) (**Figure 1E**). The proband (Case 4) was the eldest brother and presented aged 13 years with stiffness and difficulty climbing stairs. This progressed over several years until he could not walk unaided and had frequent falls. Both younger sisters (Cases 5 and 6) presented with proximal weakness aged 20 and 25 years respectively with a less severe phenotype than their brother. Their parents and two younger brothers and sister were asymptomatic. Case 5 suffered from hearing loss, cataracts, macular degeneration and reduced sensation in the distal limbs. EMG aged 47 showed prolonged, high amplitude polyphasic motor unit action potentials (MUAPs) in proximal muscle groups more than distally. She had slowed SNCV in the right median, ulnar and sural nerves with reduced SNAP in the right sural nerve. Right median and tibial MNCVs were slowed. Case 6 had initially presented with difficulty climbing stairs with cramps and frequent falls. She gradually developed proximal arm weakness. She complained of occasional burning in her hands and feet. Sensory examination revealed reduced light-touch sensation in the extremities. NCS aged 43 years showed moderate slowing in MNCV in the upper and lower limbs with low peroneal nerve CMAP. EMG revealed chronic, high amplitude, polyphasic MUAPs in bilateral upper and lower limbs.

Exome sequencing of Cases 4 and 6 from Family 4 revealed a homozygous *PLEKHG5* [c.1648C>T: NM_198681.3 (p.Gln550Ter)] variant in the Rho Guanine Exchange Factor (RhoGEF) domain. Further Sanger sequencing confirmed the same homozygous variant in Case 5. Their unaffected mother and sister were both carriers (**Figure 1E**). This variant is not reported in gnomAD, Iranome and QS IoN Database.

Family 5: Cases 7 and 8

Case 7 developed limb weakness aged 13 years. He noticed difficulty running initially and difficulty making a fist, especially in cold weather. He developed progressive difficulty walking. His asymptomatic parents were second cousins (**Figure 1F**). Examination showed proximal more than distal weakness in the arms and legs. There was no evidence of cranial nerve or sensory involvement. EMG revealed widespread chronic neurogenic process with normal NCS. A clinical diagnosis of SMA was suspected but *SMN1* analysis was normal. Since the publication of his case[8], an older affected sister (Case 8) also developed proximal limb weakness of later onset. Her EMG showed chronic neurogenic process without evidence of active denervation and decreased CMAPs.

Exome sequencing of Case 7 from Family 5 (**Figure 1F**) showed a homozygous variant in *PLEKHG5* [c.2120C>A: NM_198681.3 (p.Pro707His)]. Subsequent Sanger sequencing confirmed that his affected sister (Case 8) was also homozygous for the same variant. The affected proline residue is highly conserved and resides in the functionally-important PH domain. (**Figure 1A**). The variant is not seen in gnomAD, Iranome or QS IoN databases (**Table 2**).

Family 6: Cases 9 and 10

Two members of a non-consanguineous Turkish family (**Figure 1G**) developed proximal arm and leg weakness in adolescence. Since 13 years of age, Case 9 had difficulties getting up from crouching. She developed intermittent paraesthesia in both hands and shoulder girdles. Examination aged 30 years showed a positive left Trendelenburg's sign and Gower's manoeuvre. She had lower limb-predominant proximal weakness, lumbar hyperlordosis and was areflexic. EMG showed neurogenic changes. Muscle biopsy confirmed primary neurogenic muscle atrophy. CK was mildly elevated at 241 U/l. Her brother (Case 10) had difficulty elevating his arms with walking difficulties at 15 years of age. He developed shoulder girdle and left sternocleidomastoid muscle wasting. Unlike his sister, lower limb examination showed no weakness. Deep tendon reflexes were diminished. Sensory examination was normal. The MNCV of the peroneal and tibial nerves were 38 m/s and 40 m/s respectively and the EMG showed a predominant neurogenic pattern. His CK was 384 U/l.

For Family 6, pathogenic variants in the neuropathy gene panel (Methods) were excluded. Exome sequencing of Case 10 revealed a truncating homozygous deletion in *PLEKHG5* [c.289delC: NM_198681.3 (p.Arg97GlyfsTer38)]. Sanger sequencing verified the variant in both affected siblings and both asymptomatic parents were heterozygous carriers (**Figure 1G**). However, the homozygous single nucleotide deletion leading to frameshift and a premature stop 38 codons downstream was also identified in

their 23-year-old brother (F.6.2.5). He had not shown any signs of neuromuscular disorder to date according to his family. The variant was not found in gnomAD, Iranome, QS IoN database, or Varbank and RD-Connect GPAP Databases.

Family 7: Case 11

A 23-year-old male (Case 11) was the seventh child of consanguineous Syrian parents (**Figure 1H**). At age 10 years, he complained of shoulder weakness. He was unable to lift his arms and had difficulty climbing stairs with mild dysphagia. At 16 years of age, he developed distal arm involvement. His six older brothers and parents are healthy. Mild *pes valgus* deformity was present (**Figure 2L**). Arm and shoulder muscles were atrophic in comparison to his legs (**Figure 2M, N**). There was symmetric weakness in shoulder abduction and weakness in his forearms, wrists and hands. Mild hip abduction, adduction and plantarflexion weakness were present. Trendelenburg's sign was present. Deep tendon reflexes in the arms were absent and lower limb reflexes were diminished. Sensation was unaffected. CK was 1,100 U/l. MNCV and SNCV of right ulnar and median nerves were normal. EMG showed chronic neurogenic changes. Shoulder MRI showed muscle atrophy and fatty infiltration especially of the subscapularis (**Figure 2O-Q**). Thigh MRI showed symmetric gluteus maximus and tensor fasciae latae atrophy with prominent septal fatty tissue (**Figure 2R-T**).

We performed parent-child trio whole genome sequencing. Allele-sharing statistics confirmed parental consanguinity. A homozygous missense variant in *PLEKHG5* [c.1669A>C: NM_198681.3 (p.Met557Val)] was revealed. Sanger sequencing confirmed the homozygous variant in the patient and carrier heterozygosity in the parents (**Figure 1H**). The variant was not found in gnomAD, Iranome or any of our inhouse databases as described (**Table 2**). The methionine at residue 557 is highly conserved and located in the functionally-important RhoGEF domain (**Figure 1A**).

Family 8: Case 12

Patient 12 was born of consanguineous Indian parents and had a normal developmental history. He had progressive lower limb proximal weakness and distal muscle cramps from 8 years of age. His paternal grandfather is reported to have had dragging of his feet and so did his paternal uncle. **(Figure 1I)**. Examination aged 17 years revealed hammer toes, pes cavus and ankle contractures. He had normal cranial nerve examination, arm and thigh fasciculations, grade 4 weakness at the shoulder and hip girdles, mild distal weakness, atrophy of distal leg muscles and high-stepping, waddling gait. NCS showed normal motor

and sensory findings. Aged 42 years, distal lower limb weakness had worsened to grade 3, with moderate lower thigh atrophy and severe atrophy of leg muscles, although he continued to be ambulant (**Supplementary Video 2**). NCS showed moderate sensorimotor neuropathy (**Supplementary Table 1**). Left quadriceps muscle biopsy was characteristic of neurogenic atrophy (**Figure 1 U-X**). Exome sequencing showed a biallelic missense variant in *PLEKHG5* [c.2057C>T: NM_198681.3 (p.Thr686Met)] with a MAF of 1.42E-5 (4 heterozygous alleles out of 282,168) in gnomAD (v2.1.1) but absent in Iranome and QS IoN databases. The reference codon lies in the PH domain and is conserved across species.

Family 9: Case 13

Patient 13 is born of consanguineous Indian parents who were first cousins and had a normal birth and development. At the age of 16 years, he noticed difficulty running and experienced frequent falls. He developed progressive lower limb proximal weakness followed by distal weakness, then upper limb proximal weakness. His elder sister has similar complaints but with minimal distal leg weakness (**Figure 1J**). Examination showed right arm muscle atrophy, reduced pin-prick sensation over his hands and up to distal one-third of his legs, impaired joint-position sense at the hallux and absent deep tendon reflexes. He had a high-stepping gait. Serum CK was 1143 U/L. NCS revealed uniformly decreased MNCV and SNCV suggestive of an intermediate sensorimotor neuropathy **(Supplementary Table 1**). Exome sequencing revealed a biallelic missense variant in *PLEKHG5* [c.1364T>G: NM_198681.3 (p.Val455Gly)]. The variant is not reported in gnomAD, Iranome or inhouse databases. The reference codon for the variant lies in the functional RhoGEF domain.

Discussion

Hereditary motor neuropathies (HMN) encompass a subgroup of inherited peripheral neuropathies hallmarked by a generally more distally-pronounced, length-dependent motor neuropathy without significant sensory involvement[14]. CMT (hereditary motor and sensory neuropathy) refers to a group of heterogeneous disorders characterized by chronic motor and sensory polyneuropathy[15]. SMA comprises a group of disorders presenting with muscle weakness and atrophy from progressive degeneration of the spinal motor neuron[16]. Although many genes are shared between distal HMN and CMT type 2[15,17], *PLEKHG5* is the only known gene associated with both proximal SMA and CMT with intermediate MNCV[15,18]. The patho-mechanism of such diseases within the motor unit is not fully understood and may be related to spinal motor neuron degeneration or a more widespread peripheral motor and sensory neuropathy or both. Previous studies have shown aggregate formation in murine motor neurons

overexpressing mutant PLEKHG5 protein although the neurotoxic mechanism of these aggregates remains unknown[1]. Sural nerve biopsies in three published cases range from normal[2] in DSMA4 to severe loss of myelinated fibres in the case of intermediate CMT[3], reflecting the underlying pathogenesis.

We present a full summary of the thirteen cases in comparison with previously-reported cases of *PLEKHG5*-associated neuropathy. The majority of our cases present with either non-5q SMA or with a proximal and distal motor neuropathy (usually starting proximally), with mild sensory involvement in three families (1, 4 and 6). Two families (8 and 9) had an intermediate CMT phenotype with reduced SNAPs and more significant clinical and neurophysiological sensory involvement. The described motor neuropathy with mild sensory changes has not been previously recognised in *PLEKHG5*-associated neuropathies and may constitute a continuum between SMA and intermediate CMT, thus broadening its phenotypic spectrum. This is exemplified in Case 1, where the proximal onset of weakness and cramps, sparing of distal SNAPs with intermediate SNCV slowing are compatible with a proximal and distal motor neuropathy (previous cases of intermediate CMT had absent or reduced SNAPs). Likewise, Cases 5 and 6 present with mild sensory involvement and predominant motor presentation, consistent with a similar phenotype of a proximal and distal neuropathy, unlike in previous reported *PLEKHG5*-associated CMT where sensory changes are more profound clinically and neurophysiologically[2,3]. Lastly, Case 9 complains of intermittent sensory symptoms only with little support for neurophysiological sensory involvement. The phenotype of probands in Families 2, 3, 5 and 7 overlaps with a more pure motor involvement described by Maystadt and colleagues[1]. We also observe the presence of diffuse white matter changes on brain imaging in Case 1. Given the lack of cognitive impairment, it is unclear whether this represents incidental findings. Of note, *PLEKHG5* is expressed in both central and peripheral nervous systems[19].

We report seven novel *PLEKHG5* biallelic variants (not found in ClinVar[20] or Human Gene Mutation Database[21]) identified in our 13 cases that do not occur within the large population databases with high MAF (**Table 2**). For the missense variants (p.Pro707His and p.Met557Leu), SIFT[9] and PolyPhen[10] predictions show that both are likely deleterious and probably damaging respectively lending *in silico* support to their expected pathogenicity. For non-truncating variants, the CADD score[11] was more than 20, placing the variants among the 1% most deleterious substitutions. Segregation analyses showed convincing evidence for variant segregation with disease except the asymptomatic brother (F.6.2.5) in Family 6. As this individual is only 23-years-old, he may yet develop symptoms suggestive of disease.

We describe the same novel homozygous nonsense variant in *PLEKHG5* (p.Pro27Ter) in two Persian patients (Cases 1-2) and a Turkish patient (Case 3) who present at different ages of onset with motor-predominant neuropathies. Although this variant occurs in an alternatively spliced exon, isoforms comprising the variant-containing exon exhibit transcript-specific expression in the tibial nerve (Genotype-Tissue Expression Project v.8)[19]. Variants described in Families 4, 5, 7, 8 and 9 affect functionally-important domains. In Families 4 (p.Gln550Ter), 7 (p.Met557Leu) and 9 (p.Val455Gly), highly conserved residues are affected in the RhoGEF domain, important for *PLEKHG5*-activated RhoGEF-mediated NF-κB signalling pathway. This domain activates GTPases involved in signalling pathways regulating actin cytoskeleton dynamics, synapse formation, and neuronal survival[1,22]. Pathogenic variants in the PH domain, which regulates the RhoGEF domain independently, has been shown previously to cause lower motor neuropathy[1] and CMT[3]. The variants described in Family 5 (p.Pro707His) and 8 (p.Thr686Met) resides in the PH domain, important in NF-κB signalling.

Within our cohort, we did not discern a clear genotype-phenotype correlation, which may be due to the limited power for detection within such a rare genetic disorder. Within the same nonsense variant (p.Pro27Ter), two patients were wheelchair-bound (Cases 2 and 3) while one remained ambulatory 15 years after disease onset (Case 1). Likewise, for Family 4 (p.Gln550Ter), both sisters had a milder and later-onset phenotype than their brother. Patients with missense variants do not seem to exhibit milder disease. The two cases of intermediate CMT within our cohort were secondary to missense variants although nonsense variants were also associated with reported cases[2].

PLEKHG5-associated neuropathies present with predominant motor symptoms, usually starting proximally and can be associated with intermediate slow conduction and minor sensory involvement. We report seven novel biallelic variants in *PLEKHG5* in thirteen clinically well-characterised individuals from nine unrelated families. Individual cases are classified as non-5q SMA, intermediate CMT or a proximal and distal neuropathy by the treating neurologist. *PLEKHG5* should therefore be considered as a candidate causative gene for a wide range of hereditary neuropathies especially if motor-predominant, proximal in onset and associated with intermediate conduction velocities. Through the presentation of our cases, we extend the molecular and phenotypic spectrum to further understanding of *PLEKHG5*-associated diseases.

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Consent

Written informed consent has been provided by all patients and study subjects.

Conflicts of interest

The authors declare no conflicts of interest and individual disclosure forms of all authors are submitted with the manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure and Table Legends

Figure 1. Positions of variants and family pedigrees of cases. A. Schematic diagram showing *PLEKHG5* based on NCBI reference sequence **NM_198681.3** with numbered exons in the top panel. Note previous variants from reported cases have also been converted with reference to NM_198681.3 for consistency. The variants reported in our study are shown in red and the previous reported variants are shown in black. The middle panel represents the locations of the variants across the *PLEKHG5* protein domains. The bottom panel shows protein ortholog alignments of missense variants reported in our study and those previously reported. The asterix indicates the position of the amino acid change with darker shades indicating a more conserved sequence. Previous reported variants with the original transcripts are reported in Table 1: Azzedine *et al.*: *PLEKHG5* [c.1940T>C: NM_020631.6 (p.Phe647Ser)] and Kim *et al.*: *PLEKHG5* [c.1988C>T: NM_020631.5 (p.Thr663Met)] and *PLEKHG5* [c.2458G>C: NM_020631.5 (p.Gly820Arg)]. Panel **B**: pedigree of Family 1; Panel **C**: pedigree of Family 2; Panel **D**: pedigree of Family 3; Panel **E**: pedigree of Family 4; Panel **F**: pedigree of Family 5; Panel **G**: pedigree of Family 6; Panel **H**: pedigree of Family 7; Panel **I**: pedigree of Family 8 where ? indicates possible symptoms; Panel **J**: Pedigree of Family 9. -/- indicates a homozygous and +/- indicates a heterozygous state of the pathogenic variant.

Figure 2. Clinical and phenotypic information. Panels **A, B, C and D** relate to Case 1. Panels **E, F**, **G, H** and **I** relate to Case 2. Panels **J** and **K** relate to Case 3, Panels **L** to **T** relate to Case 11. Panels **U** to **X** related to Case 12. Panel **A** shows evidence of arm and forearm wasting on the left and Panel **B** shows evidence of scapular winging (Case 1). Panel **C** displays MRI T1 images that show evidence of moderate fatty infiltration in the thighs and calves (Case 1). Panel **D** shows evidence of diffuse deep white matter increased signal on FLAIR T2 and T2 axial MRI brain sections. Panel **E** (Case 2) shows small muscles of the hand were more severely affected on the left than on the right and bilateral arm wasting. There is evidence of severe lumbar hyperlordosis and bilateral thigh atrophy (Panels **F** and **G**). T1-weighted MRI images show evidence of fatty infiltration in the thighs (Panel **H**) more than the legs (Panel **I**). There was wasting in the distal upper limb and hand muscles **(Figure 2J, K,** Case 3). Video of examination of this patient shown in the Supplementary Video 1. Panel **L** shows evidence of relatively preserved lower limb muscle bulk and mild pes valgus deformity (**Case 11**) and Panels **M** and **N** show evidence of proximal upper limb and shoulder girdle weakness as well as shoulder girdle atrophy. Axial T2-weighted MRI of the left shoulder and upper arm shows reduced muscle bulk, partial fatty replacement of the subscapularis (red arrows, Panel **O**) and to a lesser degree of the triceps muscle (yellow arrows, Panel **P**). Coronal T1-weighted MRI of the left shoulder and upper arm shows reduced muscle bulk and partial fatty replacement

of the subscapularis muscle (orange arrows, Panel **Q**). Axial T2-weighted MRI of the thighs shows symmetric atrophy of the gluteus maximus and tensor fasciae latae muscles with prominent septal fatty tissue in both muscles (Panels **R** and **S**). T1-weighted coronal MRI of the thighs show increased septal fat in the gluteus medius and gluteus maximus muscles (Panel **T**). Panels **U-X**: Muscle biopsy findings of Case 12. Panels **U (**x100**), V** (x200): Microphotographs showing haematoxylin and eosin stain transverse sections of the muscle fibres with variation in fibre size with many angulated atrophic fibres (asterix). Scattered hypertrophic fibres are seen. Panels **W, X** (both x200): Microphotographs showing Masson's trichrome stain transverse sections of the muscle fibres showing variation in fibre size with many angulated atrophic fibres (asterix) and clumped nuclei (arrows). Scattered hypertrophic fibres are seen. Video of examination of this patient shown in the Supplementary Video 2.

Table 1. Comparison of *PLEKHG5*-associated cases. Cases reported in this article for Families 1 – 9 are reported with reference to NM_198681.3. Previously reported cases are shown as published. Hmz = homozygous, AR = autosomal recessive, N/A = non-applicable, where the investigation was not carried out. UL = upper limb, LL = lower limb. CMAP is combined motor action potential. MNCV is motor nerve conduction velocity. SNAP is sensory nerve action potential. CVs is conduction velocities. DMLs is distal motor latencies. SMA is spinal muscular atrophy and CMT is Charcot-Marie-Tooth disease.

Table 2. Variants in *PLEKHG5* (all related to NM_198681.3) and associated in silico predictions and allele frequencies. As described within the Methods: Sorting Intolerant from Tolerant (SIFT), Prediction of Functional Effect of Human nsSNPs (PolyPhen) and Combined Annotation Dependent Depletion (CADD) scores were used. Allele frequencies were interrogated through Genome Aggregation Database (gnomAD) v.21; Iranome: a catalogue of variants collated from whole exome sequencing of 800 Iranian individuals and the Queen Square Institute of Neurology (QS IoN) inhouse database comprising exome sequencing data of 15,000 individuals.N/A indicates "non-applicable".

Supplementary Data

Supplementary Table 1. Nerve conduction study results. CMAP is combined motor action potential. MNCV is motor nerve conduction velocity. SNAP is sensory nerve action potential. SNCV is sensory

nerve conduction velocity. EDB is extensor digitorum brevis. TA is tibialis anterior. Note, the reference range is only provided as a guide to aid interpretation of results for the reader and not for diagnostic purposes.

Supplementary Video 1. Examination of Case 3 aged 44 years shows evidence of proximal and distal lower limb weakness. There is evidence of wasting in the distal upper limb and hand muscles. The patient is unable to walk unaided and has a high-stepping and waddling gait.

Supplementary Video 2. Examination of Case 12 aged 42 years showing normal cranial nerve examination, proximal and distal weakness of the lower limbs, high stepping gait and wasting of the distal lower limb muscles.

	Case	Reported in this article									Previous reported cases			
		Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9	SMA	CMT Case I	CMT Case II	CMT Case III
	Reference	***Case1***	***Case 2***	***Case 3***	***Case 4, 5, 6***	***Case 7, 8***	***Case 9, 10***	***Case 11***	***Case 12***	***Case 13***	*Maystadt et al.*[1]	*Azzedine et al.*[2]	*Azzedine et al.*[2]	*Kim et al.*[3]
Genetics	**Mutation**	Hmz nonsense	Hmz nonsense	Hmz nonsense	Hmz nonsense	Hmz missense	Hmz nonsense	Hmz missense	Hmz missense	Hmz missense	Hmz missense	Hmz nonsense	Hmz nonsense	CHZ missense
Genetics	**Nucleotide change**	c.79_83del	c.79_83del	c.79_83del	c.1648C>T	c.2120C>A	c.289delC	c.1669A>C	c.2057C>T	c.1364T>G	c.1940T > C (NM_020631.6)	c.269delC (NM_198681.3)	c.1143_1149dup (NM_198681.3)	c.1988C > T, c.2458G > C (NM_020631.5)
Genetics	**Amino acid change**	p.Pro27Ter	p.Pro27Ter	p.Pro27Ter	p.Gln550Ter	p.Pro707His	p.Arg97GlyfsTer 38	p.Met557Leu	p.Thr686Met	p.Val455Gly	p.Phe647Ser	p.Pro90HisfsTer 45	p.Glu384Ter	p.Thr663Met, p.Gly820Arg
Demographics	**Sex**	Male	Female	Female	1 male, 2 female	1 male, 1 female	1 female, 1 male	Male	Male	Male	3 male, 2 female	2 male, 2 female	1 male, 1 female	Female
Demographics	**Inheritance**	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR	AR
Demographics	**Ethnic Origin**	Iran	Iran	Turkey	Turkey	Turkey	Turkey	Syria	India	India	Mali	Portugal	Morocco	Korea
Demographics	**Phenotype**	Predominant motor neuropathy	SMA	Proximal and distal motor neuropathy	Predominant motor neuropathy	SMA	SMA	SMA	Motor & sensory neuropathy	Motor & sensory neuropathy	DSMA4	Motor & sensory neuropathy	Motor & sensory neuropathy	Motor & sensory neuropathy
Demographics	**Onset (years)**	19	8	14	13 – 25	13	13 and 15	10	8	16	2 - 11.5	28 - 44	7 and 20	8
Clinical Phenotype	**Symptom at onset**	Proximal UL weakness	Proximal LL weakness	Proximal LL weakness	Proximal LL weakness	Proximal LL weakness	Proximal upper and LL weakness	Proximal UL and shoulder girdle weakness	Proximal LL weakness	Proximal LL weakness	Proximal LL weakness	Distal limb weakness	Distal limb weakness	Distal LL weakness
Clinical Phenotype	**Sensory loss**	Mild distal LL	No	No	Distal UL and LLs	No	No	No	No	Moderate distal UL & LLs	No	Yes (Distal > proximal)	Yes (Distal > proximal)	Yes
Clinical Phenotype	**Areflexia**	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Clinical Phenotype	**Weakness**	Proximal>distal	Proximal>distal	Generalised	Generalised	Proximal>distal	Proximal>distal	Proximal>distal	Distal>Proximal	Generalised	Generalised	Distal>proximal	Distal>proximal	Distal>proximal
Clinical Phenotype	**Cranial nerve involvement**	No	No	No	No	No	No	No	No	No	No	None known	None known	No
Clinical Phenotype	**Foot deformity**	None	None	None	None	None	None	None	Pes cavus / Hammer toes/ equino-cavo-varus	None	Yes	Yes	Yes	Yes
Clinical Phenotype	**Spine deformity**	None	Lumbar hyperlordosis	None	None	None	Lumbar hyperlordosis	None	None	None	Scoliosis	None	Scoliosis	No
Clinical Phenotype	**Muscle atrophy**	Left arm and forearm	Proximal > distal	Distal UL	Distal > proximal	Proximal > distal	Scapulohumeral and hip girdle	Upper proximal predominant	LL distal predominant	Right UL	Generalised	Distal upper and LLs	Distal upper and LLs	Proximal < distal
Clinical Phenotype	**Respiratory dysfunction**	None	None	None	None	None	None	None	None	None	Yes	None	None	None

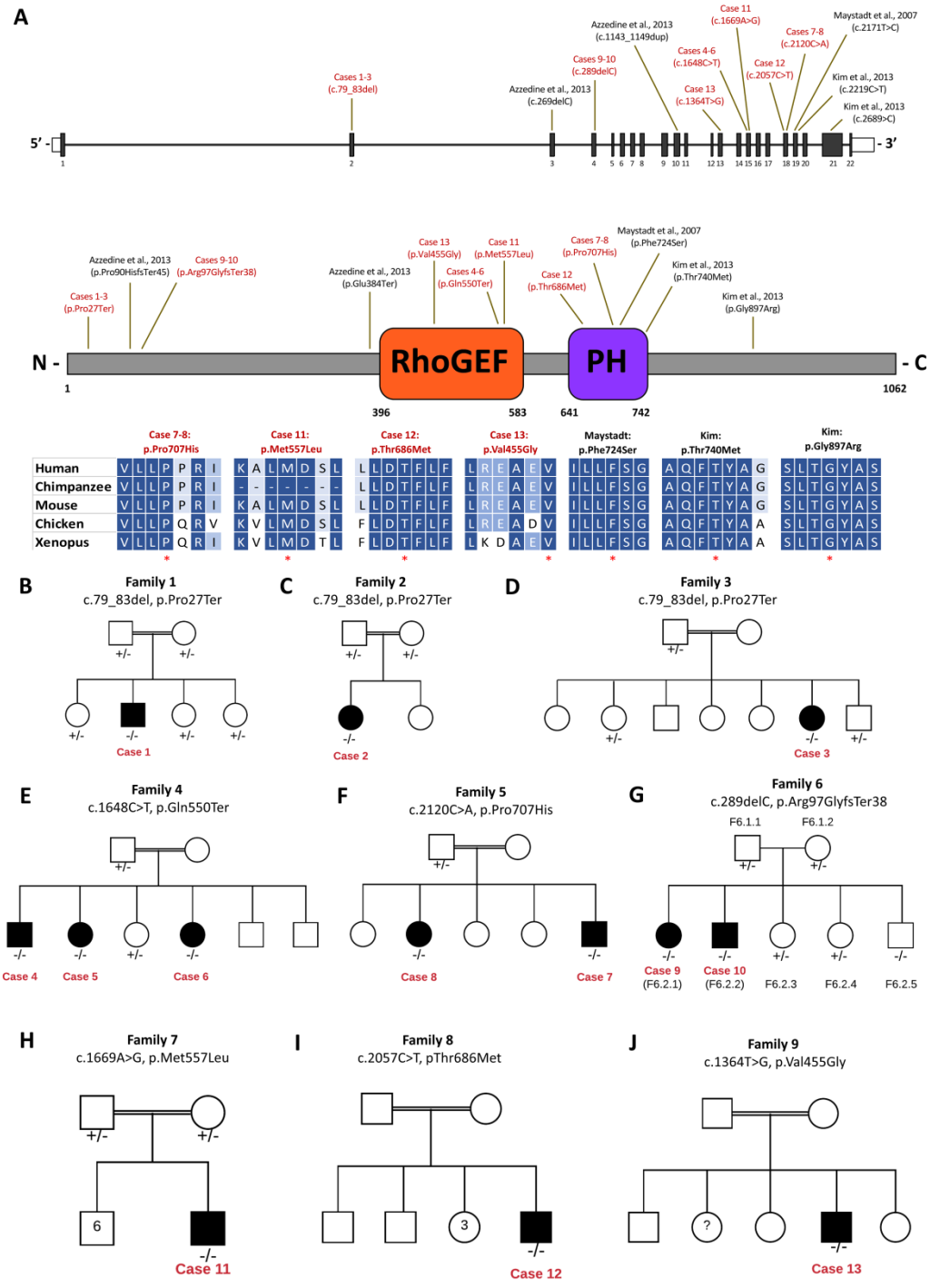
	Cardiac involvement	None	None	None	None	None	None	None	None	None	None	None	None	None
	Cortical involvement	None clinically	None	None	None	None	None	None	None	None	None	None	None	None
Investigations	**NCS**	Moderately reduced MNCV, preserved SNAPs	Reduced CMAPs, preserved SNAPs	Prolonged DMLs and reduced CMAPs	Moderately reduced MNCV, CMAP	Motor neuropathy. Reduced CMAPs	Motor neuropathy	Normal	Motor and sensory neuropathy	Motor and sensory neuropathy	Motor neuropathy	Sensorimotor neuropathy (moderately reduced CVs)	Sensorimotor neuropathy	Sensorimotor neuropathy
	EMG	Chronic process with active denervation	Chronic neurogenic changes	Chronic neurogenic changes	Chronic neurogenic changes	Chronic neurogenic changes	Chronic neurogenic changes	Chronic neurogenic changes	Neurogenic pattern	N/A	Denervation	N/A	N/A	Denervation
	Sural nerve biopsy	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Normal	N/A	Loss of large myelinated fibres, thin myelination	Severe loss of myelinated fibres
	Quadriceps muscle biopsy	N/A	N/A	Neurogenic muscle atrophy	N/A	N/A	Neurogenic muscle atrophy (Case 9)	N/A	Neurogenic muscle atrophy (Figure 2U-X)	N/A	N/A	N/A	Neurogenic muscle atrophy	N/A
	Brain MRI (age at MRI)	White matter change (33)	No abnormalities detected (19)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Normal
	Lower limb muscle MRI	Moderate fatty infiltration	Severe atrophy, fatty infiltration	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Severe atrophy, fatty infiltration

Table 1. Comparison of *PLEKHG5*-associated cases.

Cases	**Variant annotation (*PLEKHG5*)**				***In silico* predictions**			**Allele frequencies**		
	Position (hg38)	**dbSNP ID**	**cDNA change (NM_198681.3)**	**Amino acid change**	**SIFT**	**PolyPhen**	**CADD**	**gnomAD v2.1**	**Iranome**	**QS IoN**
1 – 3	chr1:6496553	rs1439392787	c.79_83del	p.Pro27Ter	N/A	N/A	28.1	1/227304 (4.4E-6)	absent	absent
4 – 6	chr1:6470860	N/A	c.1648C>T	p.Gln550Ter	N/A	N/A	41	absent	absent	absent
7 – 8	chr1:6469588	N/A	c.2120C>A	p.Pro707His	Deleterious	Probably damaging	28.3	absent	absent	absent
9 – 10	chr1:6476022	N/A	c.289delC	p.Arg97GlyfsTer38	N/A	N/A	23.7	absent	absent	absent

11	chr1:6470839	N/A	c.1669A>C	p.Met557Leu	Deleterious	Probably damaging	25.8	absent	absent	absent
12	chr1:6469651	rs553151077	c.2057C>T	p.Thr686Met	Damaging	Probably damaging	29.8	4/282168 (1.42E-5)	absent	absent
13	chr1:6471636	N/A	c.1364T>G	p. Val455Gly	Damaging	Possibly damaging	33	absent	absent	absent

Table 2. Variants in *PLEKHG5* and associated *in silico* predictions of pathogenicity and allele frequencies.



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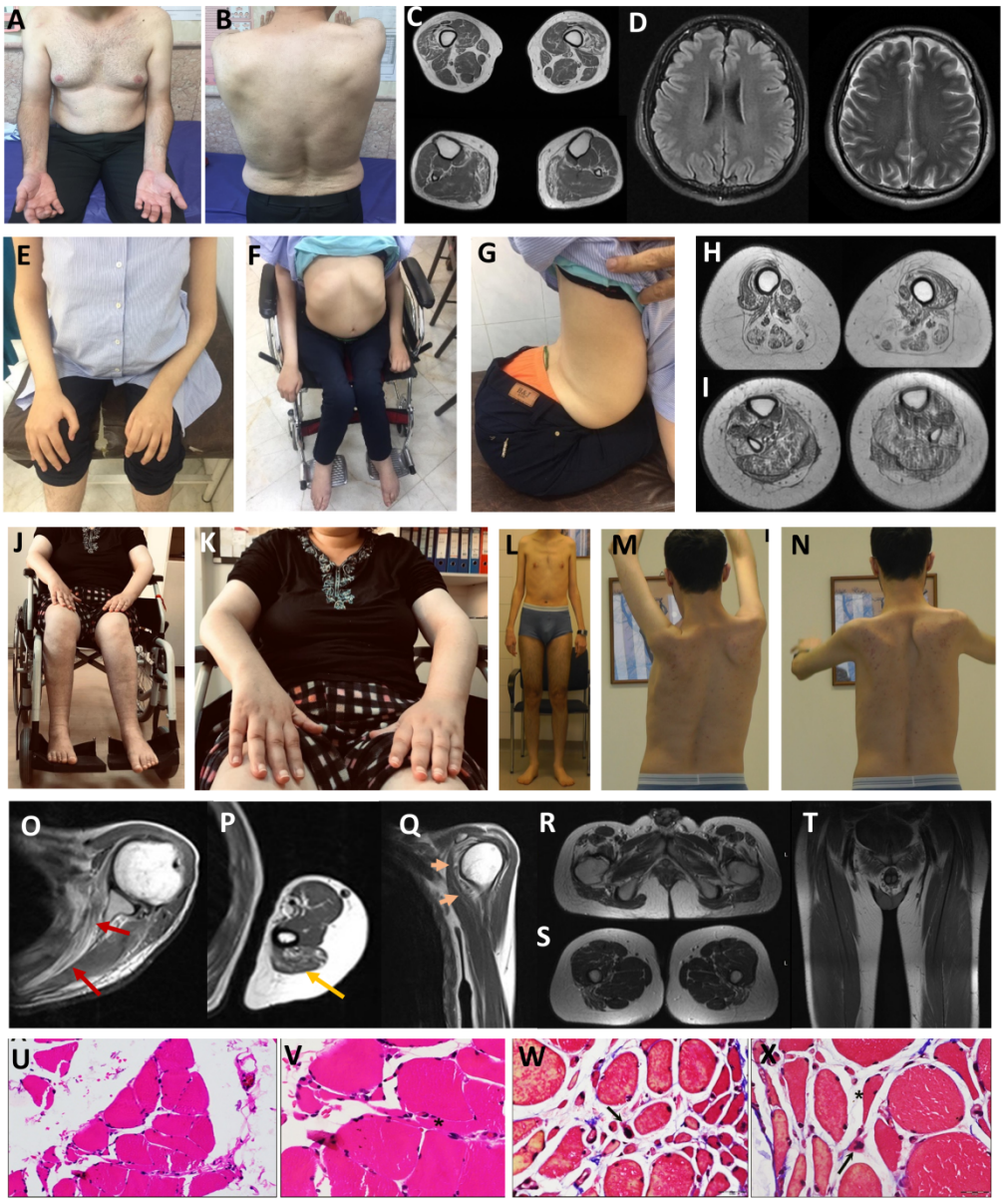

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