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Expanding the genotype-phenotype spectrum of autosomal recessive Charcot-Marie-Tooth disease: A novel *PLEKHG5* gene mutation

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Abstract

Autosomal recessive intermediate Charcot Marie Tooth (CMT) disease type C is a very rarely-seen neurogenetic disorder. Homozygous or compound heterozygous mutation in the Pleckstrin homology domain-containing family G member 5 (*PLEKHG5*) gene on chromosome 1p36 was recently reported in patients with CMT. From the first description of the disease to date, almost 40 different variants associated with the *PLEKHG5* gene were identified. Here, we present an adolescent girl who was thought initially to be myopathy because of progressive proximal muscle weakness. The electrophysiologic study revealed axonal sensory and motor neuropathy with some demyelinating features. She was diagnosed with autosomal recessive inheritance, intermediate CMT disease type C with a novel homozygous mutation in the *PLEKHG5* gene in clinical exome sequencing as *c.1600-2A>G* by next-generation sequencing. We describe here the novel mutation in the *PLEKHG5* gene and the genotype-phenotype correlation.

Keywords: PLEKHG5 gene, Charcot-Marie-Tooth disease, autosomal recessive, peripheral neuropathy

INTRODUCTION

Pleckstrin homology domain-containing family G member 5 (*PLEKHG5*) plays a major role predominantly in the peripheral nervous system, which regulates plasticity, synapse formation, dendrite growth, neuronal shape, and neuronal survival.[1] The mutations in *PLEKHG5* were reported in 2 main phenotypes, lower motor neuron disease (LMND) and intermediate Charcot-Marie-Tooth (CMT) disease.[2,3] Sensory neuropathy is the main discriminator between the 2 entities. To date, compound heterozygous and homozygous mutations in the *PLEKHG5* gene related to CMT disease were reported. Motor and sensory neuropathy in electrophysiological studies and neurological examination are characterized by progressive distal > proximal symmetric muscle atrophy and weakness, initially presenting as distal muscle weakness of the lower limbs, and foot and spine deformity were observed in almost all of the patients.[3-5] In this report, the case of a Turkish girl who was diagnosed with autosomal recessive (AR) intermediate CMT disease type-C (AR-CMTRIC) with a novel homozygous mutation in the *PLEKHG5* gene is presented.

This is a case report of a female patient in clinical follow-up at the Department of Pediatric Neurology, University of Health Sciences (SBU), Dr. Sami Ulus Training and Research Hospital, Ankara, Turkey. The patient and her legal guardians gave written consent for the publication of demographic, clinical,and laboratory data. The genetic study was performed at the Department of Medical Genetics, University of Health Sciences,

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Numune Training and Research Hospital, Ankara, Turkey, and informed consent for the genetic study was obtained from the patient and her parents. DNA was extracted using the peripheral blood of the patient. Next-generation sequencing was used with the Illumina Trusight One Panel (Trusight One, Illumina, USA). Electromyography (EMG) was performed at the Department of Neurology, Hacettepe University, Ankara, Turkey. Lower-limb magnetic resonance imaging (MRI) was performed using T1-, T2-, and STIR-weighted spin echo at the Department of Radiology of Hacettepe University.

CASE REPORT

This 8-year-old female was referred to the Department of Pediatric Neurology due to frequent falls and difficulty in climbing stairs. Symptoms had been present since the previous year but were mildly progressive. She was born at term without complications from consanguineous parents. Her prenatal, natal, and postnatal history was unremarkable. The parents reported that she had normal motor and mental development compared with her peers during infancy and childhood. The family history was unremarkable for neuromuscular diseases. On neurological examination at referral, she was weak prominently in the limb-girdle muscle. While she could barely walk on her toes and heels, she had more obvious difficulty standing up from a sitting position. Her Gowers' sign test after a duration of 10 s were positive for weakness in her limb-girdle muscles. She was able to walk without support. Deep tendon reflexes were absent. Her sensory system was intact with the evaluation of touch, position, and vibration. Creatine kinase (CPK) levels were slightly elevated at 400 ± 120 IU/L (normal range of CPK: 50–150 IU/L). Metabolic tests, such as tandem mass, urine, and serum organic acids, lactate, and pyruvate levels, were all within normal limits.

During follow-up, motor regression was observed with Gowers' sign test was positive with a longer duration. At her last visit, at the age of 10 years, she could not stand up from a sitting position without support. Neurological examination revealed muscle weakness and atrophy of bilateral distal muscles, predominantly in the lower limbs. Thoracolumbar scoliosis (25 degrees) was observed. Cerebellar and pyramidal signs and cognitive functions were within normal limits. Her cardiac evaluation, including an electrocardiogram and echocardiogram, was within normal limits. Eye examination and hearing tests were in normal ranges.

Electromyography

At her first electrophysiological examination at 8 years of age, motor nerve conduction studies (NCSs) and F-wave studies were performed for the right tibial, ulnar, and peroneal nerves, and sensory NCSs were performed for the right median, ulnar, and bilateral sural nerves using standard techniques. F-wave latencies were significantly prolonged in the median and tibial motor nerves and could not be obtained for the ulnar and peroneal nerves. The ulnar and median motor and sensory nerve conduction velocities were slow (Table 1).[6] The sural nerve sensory action potentials could not be obtained. Slight chronic neurogenic findings were determined upon needle EMG examination of the tibialis anterior muscle, and the motor unit action potentials had slightly long duration and large amplitude. The first electrophysiological study suggested sensory and motor intermediate polyneuropathy with moderate demyelinating features.

At the second electrophysiological examination at 9 years of age, F-wave abnormalities of the median and tibial nerves remained unchanged, while F-waves could be obtained for the peroneal and ulnar nerves, but with prolonged latencies (Table 1). The ulnar and median motor, and ulnar sensory nerve conduction velocities were within normal ranges, while the median sensory nerve conduction velocity was still slow.[6] Bilateral sural nerve sensory action potentials could be obtained, but with very low amplitudes. There were both significant active (fibrillation potentials and positive sharp waves) and chronic neurogenic findings for the tibialis anterior, iliopsoas, biceps, and first dorsal interosseous muscles with needle EMG examination. There were myokymic discharges in the biceps muscle. The second electrophysiological study suggested a predominantly axonal sensory and motor polyneuropathy and some demyelinating features.

Magnetic resonance imaging

Her brain MRI was normal. The MRI of the lower limbs showed a Goutallier classification of 1 degree of fatty changes in both the anterior and medial compartment muscles. Her bone structure was normal. The thickness of the subcutaneous fat tissue was significantly increased (Figure 1).

Table 1: Motor and sensory NCSs at the age of 8 and 9 years

First motor NCS at 8 years of age					Second motor NCS at 9 years of age				
Nerve	**DL (ms)**	**NCV (m/s)**	**Amplitude (μV)**	**F-wave latency (ms)**	**Nerve**	**DL (ms)**	**NCV (m/s)**	**Amplitude (μV)**	**F-wave latency (ms)**
Right median	3.1	41	6.2	30.1	**Right median**	3.6	51	5.3	29.2
Right ulnar	1.6	41	7.5	Not obtained	**Right ulnar**	2.3	51	6.1	27.5
Right tibial	2.7	40	6.4	47.2	**Right tibial**	3.2	40	6.6	49.3
Right peroneal	3.0	43	2.7	Not obtained	**Right peroneal**	3.3	40	2.8	44.2
First sensory NCS at 8 years of age					**Second sensory NCS at 9 years of age**				
Right sural	Not obtained				**Right sural**	46	2.0		
Left sural	Not obtained				**Left sural**	48	1.8		
Right median	42	23.6			**Right median**	41	31.0		
Ulnar	43	14.5			**Ulnar**	51	11.0		

DL: Distal latency, NCV: nerve conduction velocity

Genetic study

Clinical exome sequencing performed with TruSight™ One Sequencing Panel in Miseq platform (Illumina, CA, USA). The analyses of the data that included 4813 clinically relevant genes revealed a novel homozygous *PLEKHG5(NM_001265593.1):c.1600-2A>G* mutation at the canonical splice acceptor site of *PLEKHG5*. This variant was not found in any healthy controls on GnomAD data.[7] Online in-silico analyzing tools had possible pathogenic predictions including evolutionary conservation and splicing impact (DANN score: 0.9906 and GERP score: 4,53) for the variant which could lead to the distribution of the splicing of intron 14 and cause none or less-functional protein. Three more *PLEKHG5* gene splice-site variants other than the variant which is presented were reported in the Clinvar database and all of them were interpreted as likely pathogenic (rs1553174500, rs1553174566, rs144750655). However, clinical features associated with these splice-site variants were not reported. Recently, 10 cases with CMT and CMT-associated neuropathy were reported but none of the mutations were splice-site mutations.[8] Just as the mutation detected in the patient was not found in HGMD Professional (HGMD version 2020.4) and Clinvar databases, and also it was not reported in literature screening. Confirmation and segregation analyses of the variant were completed via primers designed in-house for the next-generation sequencing system for the patient and the family. It documented the carrier status of the parents and a nonaffected sibling (Figure 2a). Since the loss of function mutations in the *PLEKHG5* gene have been a well-known mechanism for the disease, all the variants in the splice region would produce the disruption of the splicing.

The interpretation of the scores in splicing effect prediction tools showed that A>G alteration likely disturbs normal splicing, as it denotes acceptor lost (MaxEntScan and ASPP (Alternative splice site programme)). Together, two in silico analyses above might imply a harmful effect on proper functioning of the splice acceptor site of *PLEKHG5* around c.1600-2A>G alteration. This variant was considered causative for the disease based on clinical concordance, segregation analysis, in-silico analyses, and the fact that this variant was not previously reported. Criteria for interpretation of the variant; a null mutation causing loss of function as it was found on the variant acceptor splice site (PVS1), according to the low frequency (PM2), and pathogenic

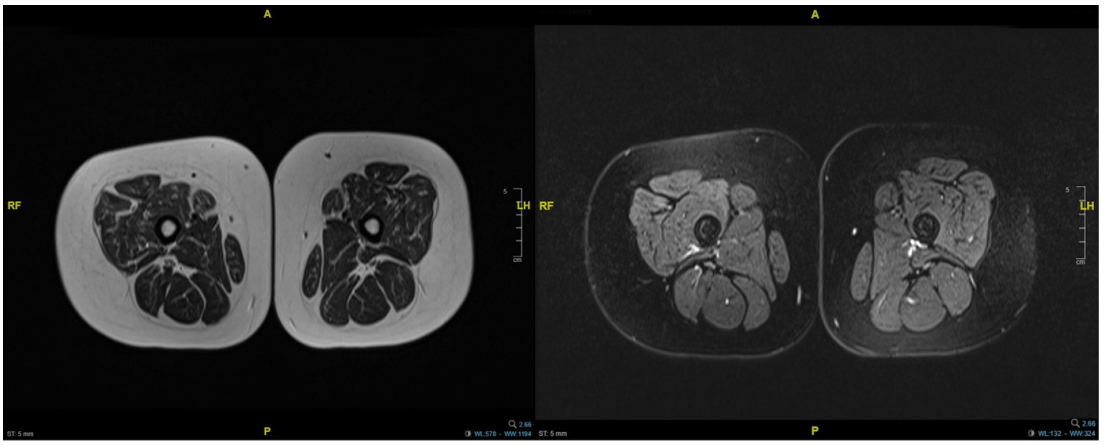

Figure 1: Lower-limb MRI showing Goutallier classification of 1 degree of fatty changes in both the anterior and medial compartment muscles.

predictions (PP3) for the variant with in-silico prediction tools; it was classified as likely pathogenic according to the Standards and Guidelines for the Interpretation of Sequence Variants.[8] Figure 2b shows the mutations of the proband and her family in IGV (integrative genomics viewer).

DISCUSSION

AR-CMTRIC related with compound heterozygous or homozygous mutations in the *PLEKHG5* gene on chromosome 1p36 was reported previously.[3,4] In the present case, a novel splice site mutation was detected, in which a null variant led to a truncated less or non-functional protein. The variant was replaced in the RhoGEF domain (a guanine nucleotide exchange factor for Rho protein) of the *PLEKHG5* protein, which contained several reported pathogenic variants. A lack of functional protein from both alleles of *PLEKHG5* was explanatory for phenotypes

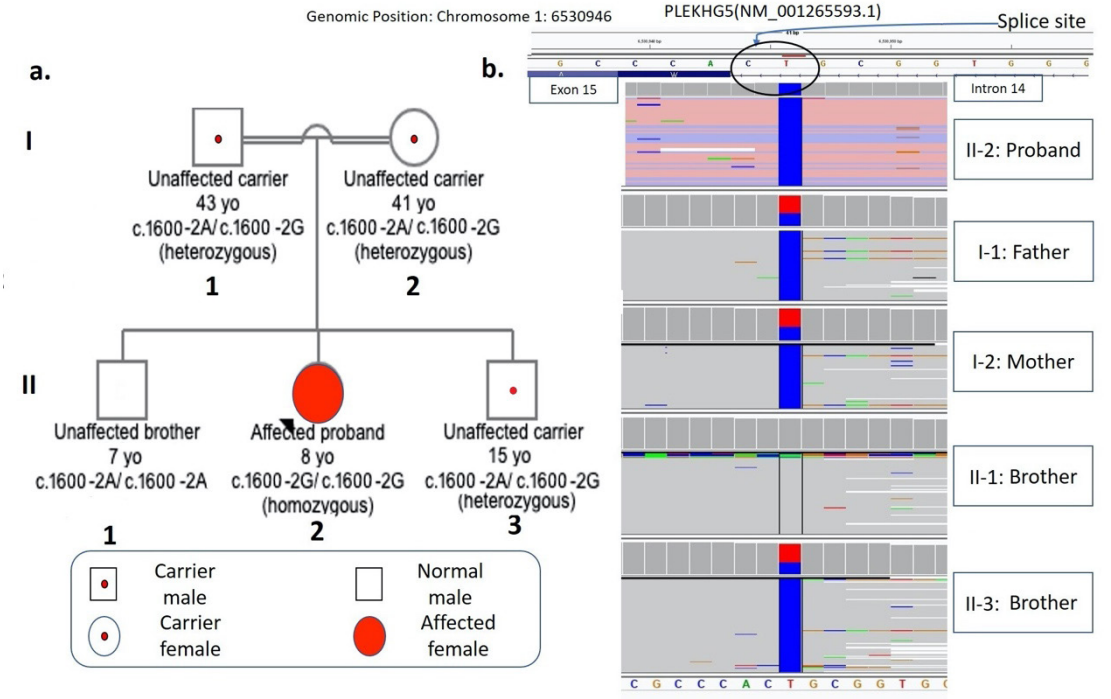

Figure **2a**. Pedigree of the family **2b**. shows the mutations of the proband and her family in IGV (integrative genomics viewer)

Table 2: Main clinical data of the reported AR-CMTRIC patients

Clinical data	Gender	Age at onset (years)	Type of mutation	Type of weakness	Distal muscle atrophy	Proximal muscle involvement	Areflexia	Distal sensory loss	Foot deformity	Spine deformity
Azzedine, 2013[3]	3M/2F	7-53	5/5 homozygous	D>P LL>UL	4/5	3/5	4/5	4/5	3/5	2/5
Kim, 2013[4]	F		Compound heterozygous	D	1/1	1/1 (LL)	1/1	1/1	1/1	1/1
Chen, 2020[5]	4F/9M	8-25	11/13 homozygous	P>D LL>UL	3/13	13/13	3/13	1/13	2/13	2/13
Laboratory data	**Muscle biopsy**	**Nerve biopsy**	**Limb MRI findings**					**NCS, EMG findings**		
Azzedine, 2013[3]	1/5 - primary neurogenic muscle atrophy (4/5 NR)	1/5- demyelinating and axonal changes	NR					NR		
Kim, 2013[4]	NR	1/1- Severe loss of myelinated fibers,	1/1- Severe muscle atrophy and fatty replacement in lower leg muscles with a selective involvement of anterior and lateral compartment muscles more than thigh.					1/1- Median MNCVs ranged from 24.7 m/s-29.3 m/s between 14-19 years of age. Prolonged motor latencies, absent SNAPs on bilateral sural nerves were revealed. Needle EMG showed neurogenic pattern.		
Chen, 2020[5]	2/13 - primary neurogenic muscle atrophy (11/13 NR)	NR	1/13 – proximal and distal involvement with moderate fatty infiltration of the medial head of the gastarocnemius, vastus lateralis, and gluteus muscles 1/13- muscle atrophy and fatty replacement 1/13- muscle atrophy and fatty infiltration in shoulder MRI, symmetric gluteus maximus and tensor fascia latae atrophy with prominent septal fatty tissue in thigh MRI					9/13 NCS- 6 of them showed motor neuropathy motor neuropathy with intermediate slow conduction velocities. NCS was normal in 3 patients with chronic neurogenic changes in EMG. 9/13 EMG - revealed chronic neurogenic process (reported as; proximal>distal in 1 patient, with active denervation in 2 patients)		

D: Distal, P: proximal, LL: lower limb, UL: upper limb, NR: Not reported, MNCVs: Motor nerve conduction velocities, SNCV: sensory nerve conduction velocity, SNAP: sensory nerve action potentials

documented in laboratory investigations, including electrophysiological, and imaging findings. There is no healthy Turkish population data for the variant reported in this case, but segregation of the variant was demonstrated by a family study. Moreover, several researchers reported that mutations in the *PLEKHG5* gene cause 2 different phenotypes, such as CMT disease, recessive intermediate C, and spinal muscular atrophy, distal, and autosomal recessive CMT.[2,4,8] Azzedine *et al*. were the first to experimentally design a mouse model lacking functional *PLEKHG5*, which mimicked the phenotypes observed in human patients.[3] The main characteristics of the reported patients are summarized in Table 2.

On admission, the case had weakness prominently in limb girdle muscle which was reported in almost all cases.[8] Distal sensory loss was reported in almost 1/3 of the patients. All of the patients had distal muscle weakness, predominantly in the lower limbs. On the contrary, weakness in the proximal muscles of the lower extremities was prominent in the patient in the current study. However, the presence of areflexia, distal muscle atrophy, and the development of spinal cord and foot deformity were consistent with other defined cases.

Electrophysiological studies of patients showed prolonged motor latencies and their median motor nerve conduction velocities were slowed (appropriate for intermediate phenotype), the amplitudes of the sensory nerve action potentials were decreased or absent, and their conduction velocities were slowed in the affected nerves. Needle EMG showed muscle denervation in almost all of the patients. In the present case, intermediate polyneuropathy was detected with moderately but predominantly demyelinating features of the sensory and motor nerves at first examination, while later axonal features became evident with active and chronic denervation findings.[5,9]

Kim *et al*. reported the involvement of anterior and lateral compartment muscles predominantly on hip MRI.[3] However, in the current study, the lower-limb MRI of the patient showed Goutallier classification of 1 degree of fatty changes in both the anterior and medial compartment muscles.

In conclusion, this mutation was not reported to date; however, mutations affecting splice-sites were reported as likely to be pathogenic in the literature. This mutation was classified as a likely pathogenic variant via American College of Medical Genetics and Genomics criteria. Moreover, the *PLEKGH5* mutation in the patient herein showed clinical and electrophysiological evidence of sensory nerve involvement, which is a key point in the diagnosis of CMT. As reported previously, the EMG findings showed axonal sensory and motor polyneuropathy with some demyelinating features. These findings helped us to classify our case as CMT rather than spinal muscular atrophy associated with *PLEKHG5* variants. Finally, this case showed a novel mutation, which will contribute to the literature in terms of genotype-phenotype correlation with clinical and electrophysiological findings.

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DISCLOSURE

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Conflict of interest: None

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