

Case Report

Report of a Patient with Limb-Girdle Muscular Dystrophy, Ptosis and Ophthalmoparesis Caused by Plectinopathy

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Abstract

Mutations in plectin, a widely expressed giant cytolinker protein can lead to different diseases mostly with signs of muscular dystrophy (MD) and skin blistering. The only report of plectin-related disease without skin involvement is limb-girdle muscular dystrophy type 2Q (LGMD2Q) phenotype, showing early-onset limb-girdle muscular dystrophy symptoms with progressive manner and no cranial muscle involvement. Here, we report a non-consanguineous Iranian family with two affected sisters showing progressive limb and ocular muscle weakness. Whole Exome Sequencing (WES) led to identification of a compound heterozygous mutations, p.Gln1022Ter (c.3064C>T) and p.Gly3835Ser (c.11503G>A), in *PLEC* gene. To the best of our knowledge, this would be the first report of a patient with LGMD and myasthenic symptoms without any skin involvement, caused by plectinopathy. This observation extends the phenotypic spectrum of *PLEC* related diseases and suggests a variable expression of the *PLEC*- related symptoms.

Keywords: Iran, limb-girdle muscular dystrophy, myasthenic symptoms, plectinopathy

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Introduction

Mutations in plectin, a widely expressed giant cytolinker protein can lead to different diseases called Plectinopathies or plectin-related diseases. These disorders include Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), EBS-MD with myasthenic symptoms (EBS-MD-Mys), limb-girdle muscular dystrophy type 2Q (LGMD2Q), EBS-pyloric atresia (EBS-PA) and EBS-Ogna (EBS-OG). Muscle symptoms are present in EBS-MD, EBS-MD-Mys and LGMD2Q. Clinical symptoms in EBS-MD mostly include blistering of the skin and progressive MD.^{1,2} There are some reports of patients showing myasthenic symptoms as well as Epidermolysis bullosa simplex with muscular dystrophy, which are called EBS-MD-Mys.^{3,4}

The only report of plectin-related disease without skin involvement is LGMD2Q (OMIM#613723) with 9bp deletion involving initiation codon in plectin isoform 1f (NM_201378). Patients with LGMD2Q phenotype showed early-onset LGMD symptoms with progressive manner and also no myasthenic features or ophthalmoplegia. Tissue-specific expression of plectin isoforms has been suggested as an explanation for phenotype of MD without skin abnormalities.⁵

Plectin is one of the cytolinker or plakin proteins, which are accessory proteins with roles in cytoskeleton stability, maintaining

integrity of cell and tissue as well as regulating the signal complexes. This giant protein is widely expressed in different tissues such as skin, muscle, heart and nerves.⁶

In this report, we describe a family with LGMD and myasthenic symptoms, who were referred to our laboratory. Whole Exome Sequencing (WES) led to identification of a compound heterozygous mutations in *PLEC* gene. To the best of our knowledge, this would be the first report of a patient with LGMD and ophthalmoplegia without any skin involvement, caused by plectinopathy and extends the phenotypic spectrum of plectin-related patients.

Case Report

Patients

The patient was from a non-consanguineous Iranian family with two affected sisters in the pedigree showing progressive muscle weakness with ptosis and dysphagia (Figure 1). The proband, a 34-year-old female, was the result of normal vaginal delivery with normal psychomotor development. She began to walk at the age of 12 months. Since the age of 4 years, her weakness has begun with progressive muscle weakness in proximal lower limbs. The muscle weakness gradually progressed to difficulty climbing stairs and occasional falling at age 7, and became wheelchair bound at age 30. Arms became weak since the age of 11 and after a while she gradually developed bilateral ptosis (Figure 1). She also mentioned slight difficulty in swallowing.

On examination, mental state was normal. She had bilateral ptosis and mild bifacial weakness with normal extraocular movements. Neck flexors weakness and scapular winging were also seen. Generalized limb muscle weakness was evident, which was more prominent in proximal muscles and arms than legs. Calf hypertrophy and scoliosis were not observed. Deep tendon reflexes were generally absent and sensory examination was normal.

It should be noted that no dermatological symptoms, especially

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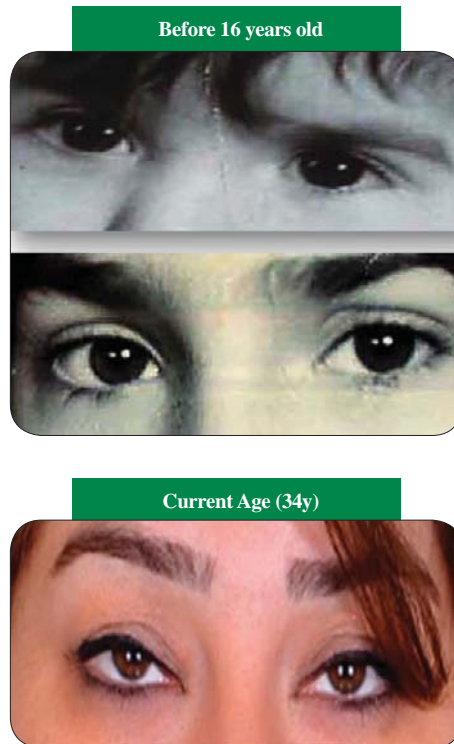


Figure 1. This figure shows ptosis observed in our proband's eye after age of 16 years.

any kind of skin blisters indicating epidermolysis bullosa simplex, nail deformity or alopecia were identified even subtle. There were no signs of respiratory complications, urinary problems or bulbar involvement. At age of 20 years, the serum creatine kinase (CK) and Lactate Dehydrogenase (LDH) levels showed significant elevation to the range up to 2000 and 700 U/L, respectively. In addition, acetylcholine receptor antibody was negative. Electro diagnostic examinations showed normal Nerve Conduction Velocity (N.C.V.) and Electromyography (E.M.G.) showed myopathic pattern 3 and 40 HZ repetitive nerve stimulation recorded from several muscles and orbicularis oculi. Single fiber electromyography (SFEMG) was negative. Echocardiogram showed normal heart function and the pulmonary function test was normal in both affected sisters.

The second affected sibling in the family was a 30-year-old female who showed a similar pattern of upper and lower limb muscle weakness, facial and pharyngeal muscle involvement, ptosis and diplopia by age of 16 years. She had a slight nasal speech, which was milder than her sister. No extra ocular ophthalmoplegia or bulbar signs were observed. Elevated serum CK up to 750 IU/L were found in her serum. NCVs were normal and EMG was myopathic. In addition, two affected sisters did not respond to anti-myasthenic medication.

Genetic Analysis

The DNA of individuals in core family was extracted from peripheral blood; after obtaining the informed consent from patients according to the ethical committee recommendations. The 34-year-old female proband was selected for whole exome sequencing (WES) with the means of Agilent SureSelect Human Exome Kit (V4) for capturing sequence target regions and paired-end sequencing for 100 cycles (generating 100 bp reads) on one third lane of Illumina HiSeq2000's flow-cell.

Analysis was performed with more focus on variants within 62 selected known MD genes, involving LGMDs, Congenital muscular dystrophies, Distal muscular dystrophies (distal myopathies), Oculopharyngeal, and Emery-Dreifuss muscular dystrophy as well as Duchenne and Becker muscular dystrophy.

No candidate homozygous mutation among the 62 selected known genes was identified. Considering that the patient's parents were not consanguine, the probability of compound heterozygous mutation was increased; therefore the candidate heterozygous variants were selected (Table 1). Confirmation of all the detected variants as well as co-segregation study in the family was performed with the help of conventional Sanger sequencing resulted in identification of compound heterozygous mutations p.Gln1022Ter (c.3064C>T) and p.Gly3835Ser (c.11503G>A) as the cause of phenotype in the family.

Discussion

The *PLEC* gene, located on 8q24, encodes a multi domain protein consisting two Globular domains located in N- and C-terminals which are connected by central α -helical coiled-coil Rod domain. The central Rod domain where the dimerization occurs is encoded by exon31, which predominantly harbors mutations in EBS-MD patients. The constant section of the globular N-terminal domain, recognized as the actin binding domain of the protein, constitutes a highly conserved ABD and a plakin domain. Whereas the globular C-terminal domain, encoded by the last exon, includes six highly homologous plectin repeat domain (PRDs) and has been recognized mostly as the IF-binding region of the giant protein.⁶⁻⁹

Our two patients came from the same family with LGMD along with later development of ocular symptoms. Whole exome sequencing revealed three novel heterozygous variants;

Table 1. List of the final candidate heterozygote variants

Variant	Gene	Classification	HGVS Coding/Protein	Location in the protein	dbSNP (MAF)	Bioinformatics predictions (PolyPhen/SIFT/Mutation Taster)	Co-segregation status in the family	Ethnic specific normal study (All Homo Hetero)
Chr2:179439823 C>T	<i>TTN</i>	Coding	(NM_001267550) c.71036G>A p.Arg23679Lys	WD 8 Repeat	rs200144345 (0.0251)	Probably Damaging/ Damaging/ Disease Causing	Co-segregates	285 0 1
Chr2:179634421 T>G	<i>TTN</i>	Coding	(NM_001267550) c.8887A>C p.Thr2963Pro	Ig-like 16 Domain	rs200875815 (NA)	Probably Damaging/ Damaging/ Disease Causing	Does not Co-segregate	285 15 153
Chr8:144992567 C>T	<i>PLEC</i>	Coding	(NM_000445) c.11503G>A p.Gly3835Ser	C-terminal globular domain	NA	Probably Damaging/ Damaging/ Disease Causing	Co-segregates	285 0 0
Chr8:144998495 G>A	<i>PLEC</i>	Coding	(NM_000445) c.5683C>T p.Arg1895Trp	Central fibrous rod domain	rs200543521 (0.0685)	Probably Damaging/ Damaging/ Disease Causing	Co-segregates	285 0 0
Chr8:145003680 G>A	<i>PLEC</i>	Coding	(NM_000445) c.3064C>T p.Gln1022X	N-terminal globular domain	NA	NA/ NA/ Disease Causing	Co-segregates	285 0 0

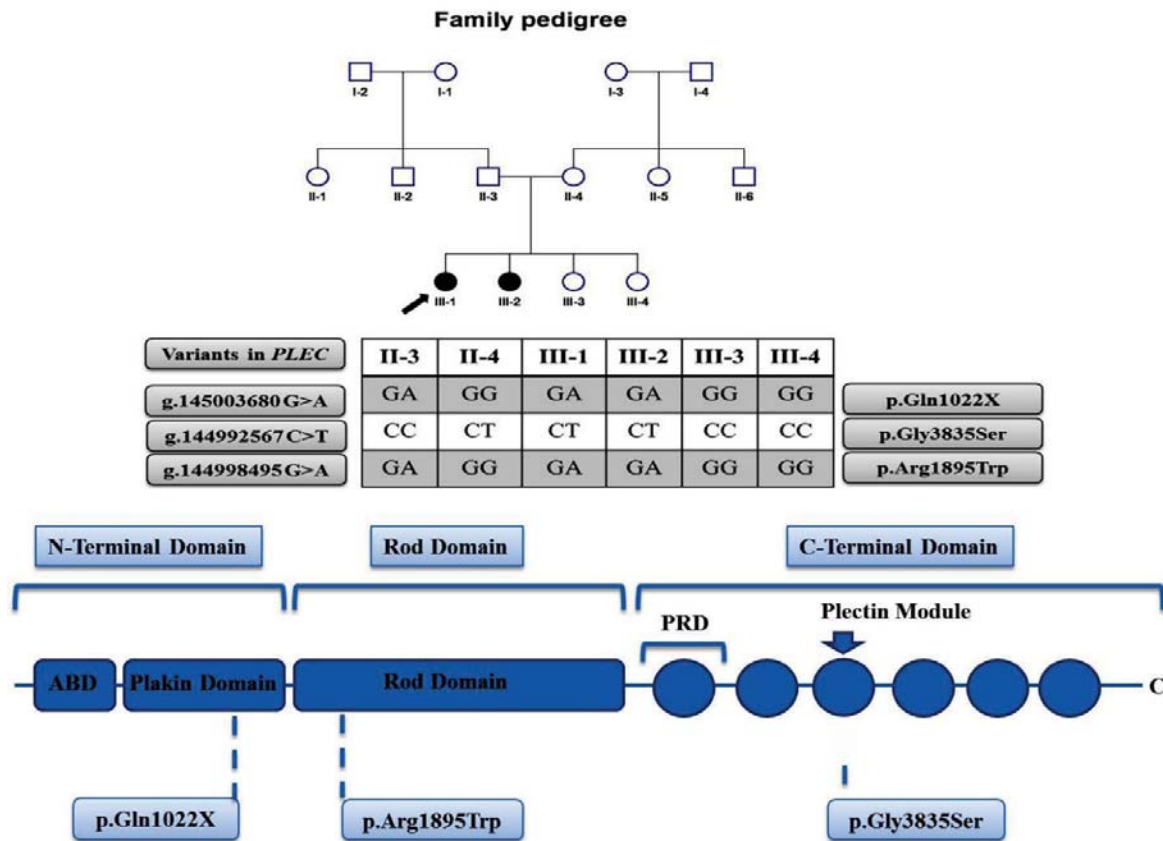


Figure 2. Pedigree of the family in this study. Co-segregation results of the three identified variants in *PLEC* gene as well as approximate location in the protein are indicated in the picture.

p.Gln1022Ter (c.3064C>T), p.Arg1895Trp (c.5683C>T) and p.Gly3835Ser (c.11503G>A) located in Plakin domain of the N-terminal, Rod domain and PRD of the C-terminal domain, respectively. All these variants co-segregate with the phenotype in the family (Figure 2). Clearly, according to the allele distribution among the parents there are two possible combinations of compound-heterozygous mutations as the cause of phenotype; p.Gln1022Ter and p.Gly3835Ser or p.Gly3835Ser and p.Arg1895Trp. Both missense variants are predicted to be pathogenic by dbNSFP.¹⁰ However, p.Arg1895Trp was seen in 7 out of total 5,107 subjects of NHLBI exome sequencing data,¹¹ while p.Gly3835Ser was not seen in any of NHLBI exome sequencing data in addition to 285 Iranian healthy controls. Based on that, it is more likely that p.Gln1022Ter and p.Gly3835Ser compound heterozygous mutations are responsible for the observed phenotype in this family.

As mentioned previously, there were no dermatological symptoms, including erosions or blistering over the entire body of our patients, which are typically one of the features of *PLEC* mutations. Gundesli, et al. reported patients with LGMD2Q phenotype; showing early-onset LGMD symptoms with progressive manner, no myasthenic features or oculo-bulbar weakness and no skin involvement. Tissue-specific expression of plectin isoforms was suggested as an explanation for the observation of phenotype with MD without skin abnormalities.⁵ In the present study, limbs and ocular weakness without skin involvement was observed in the patient, which makes it distinct from the previous report of LGMDQ. The compound heterozygous mutations p.Gln1022Ter

(c.3064C>T) and p.Gly3835Ser (c.11503G>A) identified in our patient are located within N-terminal and C-terminal globular domains and could be identified in all *PLEC* isoforms and not only in *PLEC1f*; the tissue-specific isoform in muscle. It should be noted that, Forrest, et al. (2010) reported a patient with congenital muscular dystrophy, late-onset myasthenic symptoms and only subtle dermatological signs which expanded the associated phenotypic spectrum of this gene. They concluded that even in the absence of prominent skin involvement, *PLEC* could be considered as the responsible gene for the phenotype. This might explain the observation of no erosions or blistering in our patient, although compound heterozygous mutations are located in all isoforms of the plectin. However, the presence of modifier factor cannot be rejected which remains to be more investigated.⁴

Considering the clinical symptoms, the first clinical impression of these patients was familial myasthenia, Limb-Girdle (OMIM#254300) which is considered as one type of congenital myasthenic syndrome (CMS) with wide range of clinical heterogeneity. Ptosis, ophthalmoparesis, facial weakness, and proximal muscle weakness which are compatible with the syndrome were observed in our patient. But, exome sequencing revealed no causal mutation in the *AGRN*, *DOK7* and *GFPT1*, the three genes which have been reported in this syndrome. However, our patients did not show other signs of this syndrome including; respiratory muscle weakness and abnormal neuromuscular transmission. These all together could be considered as strong indication for the phenotype caused by defects in plectin protein.^{12, 13}

In conclusion, our findings indicate that although LGMD with

ocular features has been observed along with EBS so far;¹⁴ plectin mutations can cause this phenotype even in the absence of skin involvement. This observation extends the phenotypic spectrum of *PLEC* related diseases and suggests a variable expression of the *PLEC*-related symptoms.

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Conflict of Interest: None.

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