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# Nadir Bir Hastalık Olan Lenfatik Malformasyon 6: İki Yeni *PIEZO1* Varyantı ve Literatür Taraması

# A Very Rare Disease of Lymphatic Malformation 6: Two Novel *PIEZO1* Variants and Review of The Literature

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**Öz**

**Giriş ve Amaç:** Lenfatik malformasyon 6 (MIM no: 616843) yaygın ödem, bilateral plevral efüzyon, asit ve non-immunhidrops fetalis ile karakterizedir. *PIEZO1* geni varyantları bu hastalık ile ilişkilendirilmektedir. Bu çalışmada generalize lenfatik displazinin çeşitli klinik prezantasyonları ile başvuran olguların değerlendirilmesi amaçlanmıştır.
**Gereç ve Yöntemler:** 2015 Ocak–2021 Ocak tarihleri arasında 5 olgu generalize lenfatik displazi ön tanısıyla değerlendirildi. İndeks vakalara tüm ekzom dizileme, aile bireylerine sanger dizileme analizi yapıldı.
**Bulgular:** İlk ailede yeni bileşik heterozigot *PIEZO1* geni: NM_001142864.4: c.4030_4032delGAG (E1344del)/ c.5455_5456delAA (K1819Efs*46) varyantı, ikinci ailede yeni bir homozigot *PIEZO1* geni: c.5876A>G (D1959G) varyantı tanımladık.
**Sonuç:** Beş hastanın uzun sürede klinik özelliklerini ve varyasyonlarını sunan bu çalışma, oldukça nadir bir hastalık olan Lenfatik Malformasyon 6'nın fenotip ve mutasyon spektrumuna katkıda bulunmuştur. Yeni bileşik heterozigot E1344del/K1819Efs*46 varyasyonu, erken durdurma kodonuna yol açararak daha kısa bir PIEZO1 protein ürününe neden oldu. İki bileşik nonsense varyasyonun, aile 1'in kötü prognozu ile ilişkili olabileceği düşünülmüştür. Ayrıca, ikinci yeni c.5876A>G (D1959G) homozigot mutasyonu fonksiyon kaybına neden olarak bozuk PIEZO1 protein üretimi ile ilişkili olduğu düşünülmüştür. Ayrıca, bu çalışma Türk popülasyonunu içeren ilk Lenfatik malformasyon 6 bildirimidir.

**Anahtar Kelimeler:** Lenfatik malformasyon 6, *PIEZO1* geni, Tüm ekzom dizileme, Yeni varyantlar.

**Abstract**

**Objective:** Lymphatic Malformation 6 (MIM no:616843) is characterized by generalized edema, bilateral pleural effusion, ascites and non-immune hydrops fetalis. *PIEZO1* gene has been associated with this disorder. Here, we aimed to evaluate cases with various clinical presentations of generalized lymphatic dysplasia.
**Materials and Methods:** Between January 2015 and January 2021, 5 cases were evaluated with a pre-diagnosis of generalized lymphatic dysplasia. Whole exome sequencing was performed on the index cases, and family segregation analysis was performed by Sanger sequencing.

**Results:** We identified a novel compound heterozygous *PIEZO1* gene: NM_001142864.4: c.4030_4032delGAG (E1344del)/ c.5455_5456delAA (K1819Efs*46) variants in the first family and a novel homozygous *PIEZO1* gene: c.5876A>G (D1959G) variant in the second family.
**Conclusion:** This study, which presents the clinical features and variations of five cases with long-term follow-up, contributed to the phenotypic and mutation spectrum a very rare disease of Lymphatic Malformation 6. The novel compound heterozygous E1344del/K1819Efs*46 variation leads to a premature stop codon that caused a shorter PIEZO1 protein product. It was thought that two compound nonsense variations might be associated with the poor prognosis of family 1. The second novel c.5876A>G (D1959G) homozygous mutation was thought to be associated with impaired PIEZO1 by causing loss of function. Also, this study was the first LMPHM6 report from the Turkish population.

**Keywords:** Lymphatic malformation 6, Novel variants, *PIEZO1* gene, Whole exome sequencing.

## 1. Introduction

Lymphatic Malformation 6 (LMPHM6 [MIM no: 616843]) is characterized by swelling of the face, neck and/or whole body, bilateral pleural effusions, pericardial effusion, ascites, cellulitis and non-immune hydrops fetalis (NIHF) [1]. The *piezo-type mechanosensitive ion channel component 1* (*PIEZO1* [MIM no:611184]) gene variations cause LMPHM6 disorder. *PIEZO1* gene is mapped to *16q24.3* region and consists of 51 exons. The PIEZO1 protein plays a central role in mechano-transduction in the cardiovascular, renal and hematopoietic systems [2]. The LMPHM6 sub-type was first described by Fotiou et al. in 2015 [3]. An association between perinatal edema and *PIEZO1* gene variation was first demonstrated by Andolfo et al. in dehydrated hereditary stomatocytosis (DHS), but this disease has an autosomal dominant inheritance pattern. Variations in the PIEZO1 gene have been associated with two different diseases [4]. Heterozygous gain-of-function variations in the PIEZO1 gene result in increased erythrocyte membrane permeability and lead to DHS 1, with or without pseudohyperkalemia and/or perinatal edema (MIM no:194380). Homozygous/ compound heterozygous loss of function variations in the *PIEZO1* gene is the cause of LMPHM6 (MIM no:616843).
In this report, we aimed to examine five patients from two different families with various clinical findings of generalized lymphatic dysplasia, mainly in terms of genotype-phenotype correlation with the literature. In addition, we presented two novel *PIEZO1* gene variants.

## 2. Materials and Methods

*2.1 Patients*

Between January 2015 and January 2021, 5 cases were evaluated with a pre-diagnosis of generalized lymphatic dysplasia.

*2.1.1 Family#1*

The first family consists of a 44 years-old man and an unrelated 39 years-old woman, with five consecutive pregnancies, (gravidity 5, parity 2), all of which resulted in either fetal or neonatal death.

*2.1.2 Family#2*

The index case is a male premature baby who was born at 29 gestational weeks. He died after delivery. His parents were a consanguineous, healthy Turkish couple. This patient was being followed up since the 16th gestational week due to NIHF. There was no additional anomaly seen on obstetric USG.

*2.2 Statement of Ethics*

This study was conducted in Manisa Celal Bayar University Medical Faculty Health Sciences. Ethical approval was obtained from the institutional Ethics Committee, dated 02/06/2021 and numbered 20.478.486/843. Informed consent was obtained from all patients. Protocols compatible with the Declaration of Helsinki were used in this study.

*2.3 Genetic testing*

Peripheral venous blood was collected from the second daughter of the first family. In the second proband, umbilical cord samples were taken from a male baby who was born with NIHF as the second child of a first-degree cousin mother and father. Genomic *DNA* (gDNA) was isolated from peripheral blood samples of patients according to the QIAamp Blood kit protocol (Qiagen, Hilden, Germany). Whole Exome Sequencing (WES) was performed by the capture of the coding regions and splice sites of targeted genes using the Illumina SureSelect V6 Exome kit (Agilent, Inc.). After library enrichment and quality control, the samples were sequenced using the Illumina (HiSeq4000) instrument with 100bp paired-end reads at an average sequencing depth of 100x. WES was performed on only two probands from each family and subsequent family segregation analysis was performed using ABI 3130 Sanger sequencing (Applied Biosystems Inc.). Family segregation was performed by designing primer sets specific to the variants found in patients.

*2.3.1 Analysis of next-generation DNA sequencing data*

Pathogenic variants associated with clinical features were filtered by following steps, respectively: 1) all missense, nonsense, frameshift, frame and synonymous variants, 2) variants with minor allele frequency <1.0% in population studies [1000 Genomes (1000G), ESP, ExAC and the Genome Aggregation Database (gnomAD)]. New variants in the HGMD® and ClinVar (http://ncbi.nlm.nih.gov/clinvar) databases were checked. Pathogenicity scoring of novel variants was performed according to the criteria of the American College of Medical Genetics and Genomics (ACMG) [5].

## 3. Results and Discussions

*3.1 Results*

*3.1.1 Family#1*

The first female baby of the family, III.3, was diagnosed with bilateral pleural effusion and an intrauterine thoracic-amniotic shunt was placed at 20 weeks. She was delivered by Cesarean section at 34 weeks. She died on the first postpartum day due to respiratory failure.

The second female baby, III.4, was also diagnosed with bilateral pleural effusion and the thoraco-amniotic shunt was placed at 16 weeks. Cesarean delivery was performed at 35 weeks and the date of birth was 22nd of September 2012. The birth weight of the baby was 1250 g (<3rd percentile), the length was 45 cm (<3rd percentile), and the head circumference was 32 cm (<3rd percentile). Edema has become evident in the face and lower extremities after birth. She was intubated due to pneumothorax and respiratory distress in the neonatal intensive care unit. Bilateral atelectatic lung parenchyma and pleural effusion were detected on the thorax USG. Abdominal and transfontanelle USG detected no significant pathology. She died at six months of age due to respiratory failure. We found the novel compound heterozygous variant *PIEZO1* gene: NM_001142864.4: E1344del (c.4030_4032delGAG) and K1819Efs*46 (c.5455_5456delAA) from the proband of the first family, III.4, on 18th of September 2015. Chromosome analysis from peripheral blood showed a 46, XX.

The third pregnancy, III.5, was terminated due to NIHF at 16 gestational weeks. This individual also had the same *PIEZO1* gene variant.

The fourth pregnancy, III.6, was spontaneously aborted at 12 gestational weeks. In this case, a single umbilical artery was observed on obstetric USG.

The fifth pregnancy of this family, III.7, had a similar obstetric history to the others, and bilateral pleural effusion was detected via obstetric ultrasound at 17 weeks. Although a thoraco-amniotic shunt was inserted at 18 gestational weeks, edema progressed and prognosis deteriorated and intrauterine fetal death occurred due to NIHF in the 20th week. On the 30th of April 2016, karyotype analysis of individual III.7, obtained by amniotic fluid sample, was evaluated as normal. This individual also had the same *PIEZO1* gene variant.

These E1344del (c.4030_4032delGAG) and K1819Efs*46 (c.5455_5456delAA) variations are inherited from the mother and the father respectively.

*3.1.2 Family#2*

Karyotype analysis from cultured umbilical cord sampling revealed a normal male karyotype, 46, XY. We identified a novel homozygous variant, D1959G (c.5876A>G) in this patient on 15th May 2020. His mother was heterozygous for the D1959G (c.5876A>G) variation.

*3.2 Discussion*

In this report, five patients were diagnosed with the very rare LMPHM6 disease. Two novel variants in the *PIEZO1* gene were identified, NM_001142864.4: K1819Efs*46 (c.5455_5456delAA) and D1959G (c.5876A>G). These variations have not been previously reported in variant types observed at this position in sources including LOVD (https://www.lovd.nl) and HGMD (http://www.hgmd.cf.ac.uk/ac/all.php).

Evidence from The Human Gene Mutation Database (HGMD® Professional 2020.2) suggests that homozygous or compound heterozygous variants in the *PIEZO1* gene have been primarily associated with lymphatic dysplasia with non-immune hydrops (NIHF), lymphatic dysplasia alone, or NIHF alone [1-3, 6]. Additionally, Yates et al., in a study of 84 fetal WES cases with ultrasound (USG) anomaly resulting in fetal death or pregnancy termination, reported a homozygous *PIEZO1*: p.Glu679Ter variant in a fetus whose main USG finding was hydrops [7]. Vora et al., performed exome sequencing in 15 fetuses with congenital anomalies whose karyotypes and microarray analyzes were normal, and they reported a compound heterozygous variant in the *PIEZO1* gene: c. 307C>T: p. Arg103Ter/ c.7129+1G>C in a fetus with NIHF [8]. Shamseldin et al., performed a molecular autopsy on unexplained fetal deaths with WES and detected a homozygous *PIEZO1*: c.1264C>T: p.Gln422Ter variant associated with the NIHF phenotype in their study [9]. In 2020, Sparks et al., performed a whole exome analysis to elucidate the etiology of 127 NIHF cases. Among these cases, they found a compound heterozygous *PIEZO1*: p.Pro1906Lysfs*55/p.Ile2270Thr variant in a patient with the generalized lymphatic disorder [10]. In another recent study by Guo et al., trio WES was performed on 40 unrelated families who experienced fetal death with unexplained recurrent fetal malformations and six different *PIEZO1* gene variations were reported, including four missense, one small deletion and one small insertion type [11].

The heterozygous p.E1344del (c.4030_4032delGAG) alteration that we found in family#1 resulted in in-frame deletion by causing three nucleotide deletions in exon 28 of the *PIEZO1* gene. This variation was reported by Chen et al. in a case with NIHF as a compound heterozygous with the p.Arg1299Cys (c.3895C>T) variant [12]. The other heterozygous K1819Efs*46 (c.5455_5456delAA) variation that we found in family#1 causes a frameshift variant as two nucleotide deletions occur in exon 39 of the PIEZO1 gene. According to the new reading frame, after position 46, an abnormal stop codon occurs and encodes a truncated protein. The homozygous missense variation D1959G (c.5876A>G) that we found in family#2 causes aspartic acid to be replaced with glycine within exon 41 of the *PIEZO1* gene. Glycine conveys molecular flexibility and can disturb the required rigidity of the protein at this position. This variation converts the wild-type residue charge from negative to neutral [13]. These three variations, p.E1344del, K1819Efs*46 and D1959G, are defined as likely pathogenic (PM2, PM4, PP3, PP5), likely pathogenic (PVS1, PM2) and of uncertain significance (PM2, PP3), respectively, according to the American

College of Medical Genetics and Genomics (ACMG) guidelines criteria. These variations are located in a conserved area among other species. To date, 28 single nucleotide variants associated with this disease have been identified, according to Clinvar. Our case has the worst prognosis among patients reported to date, resulting in two neonatal deaths and two prenatal deaths. Even among patients with the same variation, although there is a difference in the age of onset and clinical signs of the disease, cases in the first family are a clinical progression that begins with bilateral pleural effusion at approximately gestational week 16 in all cases.

## 4. Conclusion

In this study, two novel variants in the PIEZO1 gene associated with the very rare LMPH6, together with genotype-phenotype correlations were presented. It is very important to clarify the etiology in cases with congenital lymphedema in terms of predicting the progression and determining the most effective treatment option. Prenatal preimplantation genetic testing for a healthy pregnancy opportunity was offered to these families. Interestingly, our cases were the first report of the Turkish population in this region.

## 5. Acknowledgement and Disclosures

The authors have no conflicts of interest to declare.
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