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CLINICAL REPORT

Identification of novel *PIEZO1* variants using prenatal exome sequencing and correlation to ultrasound and autopsy findings of recurrent hydrops fetalis

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Nonimmune hydrops fetalis (NIHF) is a rare disorder with a high perinatal mortality of at least 50%. One cause of NIHF is generalized lymphatic dysplasia (GLD), a rare form of primary lymphedema of the extremities and systemic involvement including chylothoraces and pericardial effusions. An autosomal recessive form of GLD has been described, caused by variants in the *PIEZO1* gene. It has been reported clinically to cause NIHF and childhood onset of facial and limb lymphedema, most of which were diagnosed postnatally. We present a case of a woman with recurrent pregnancies affected by NIHF because of novel compound heterozygous variants in the *PIEZO1* gene diagnosed prenatally using exome sequencing (ES). Two variants in *PIEZO1* (c.3206G>A and c.6208A>C) were identified that were inherited from the father and mother, and are predicted to cause a nonsense and missense change, respectively, in the *PIEZO1* subunits. Ultrasound demonstrated severe bilateral pleural effusions, whole body edema and polyhydramnios. Histopathology revealed an increased number of lymphatic channels, many of which showed failure of luminal canalization. Sanger sequencing confirmed the same variants in a prior fetal demise. We provide phenotypic correlation with ultrasound and autopsy finding, review *PIEZO1* variants as a cause of GLD and discuss the uses of prenatal ES to date.

KEYWORDS

exome sequencing, generalized lymphatic dysplasia, hydrops fetalis, *PIEZO1* variants, prenatal diagnosis

1 | INTRODUCTION

Nonimmune hydrops fetalis (NIHF) is a rare disorder (1 of 4,000 births; Steurer et al., 2017) with a high perinatal mortality of at least 50% (Santo et al., 2011). It has many etiologies including chromosomal abnormalities, cardiac anomalies, infections, anemia, and disorders of the lymphatic system (Norton, Chauhan, & Dashe, 2015). Generalized lymphatic dysplasia (GLD) is a rare form of primary lymphedema of the extremities and systemic involvement including pleural effusions, chylothoraces, and pericardial effusions. An autosomal recessive form of GLD has been described, caused by variants in the genes *CCBE1* (Alders et al., 2009), *FAT4* (Alders et al., 2014), *ADAMTS3* (Brouillard et al., 2017), and *PIEZO1* (Fotiou et al., 2015). This has been reported clinically to cause childhood onset of facial and limb lymphedema, most of which were diagnosed postnatally (Fotiou et al., 2015). *CCBE1* and *PIEZO1* have also been reported as causes of prenatally identified NIHF (Fotiou et al., 2015). Prenatal diagnosis of GLD is rare, given the inability to identify single gene disorders on standard prenatal diagnostic techniques including karyotype and chromosomal microarray analysis (CMA). Exome sequencing (ES) is a technique using next-generation deoxyribonucleic acid (DNA) sequencing that is increasingly utilized prenatally to identify mutations in fetuses with anomalies. In the setting

of an anomalous fetus, karyotype and CMA will provide an explanation in 40% of cases. A recent review has shown that ES will reveal pathogenic variants in an additional 30% of cases after a normal CMA and karyotype (Best et al., 2017). Regarding NIHF specifically, it is estimated that up to 14% are because of a single gene disorder that would not be picked up by conventional methods (Wapner, Brenna, & Bier, 2017). Here, we present a case of a woman with recurrent pregnancies affected by NIHF because of compound heterozygous variants in the *PIEZO1* gene diagnosed prenatally using ES.

2 | CLINICAL REPORT

A 35-year-old woman presented at 23w4d of gestation with recurrent NIHF noted on ultrasound. Her obstetrical history was significant for two prior pregnancies affected by intrauterine fetal demise (IUFD). The couple's first pregnancy, II-A in Supporting Information Figure S1, was complicated by a fetal demise at 36 weeks in the setting of scant prenatal care, and no subsequent workup was performed. For their second pregnancy, II-B, the couple presented with a fetus at 22w2d with scalp edema and large bilateral pleural effusions. Amniocentesis revealed a normal female CMA and she underwent thoracoamniotic shunt placement at 27 weeks. She ultimately presented with an IUFD at 32 weeks and on autopsy was found to have chorioamniotic membrane separation with subsequent umbilical cord strangulation by the fetal membranes, thought to likely be a complication of her antecedent fetal surgery (Han et al., 2017). Of note, the patient had two prior terminations and two prior full-term live children with a different partner.

During her case pregnancy, II-C, a thickened nuchal fold raised suspicion for fetal hydrops at 16 weeks. Thorough NIHF workup was again performed and revealed Rh-positive blood type and negative serum studies for syphilis, parvovirus, and toxoplasmosis. She had normal structures on anatomic survey, normal fetal echocardiogram, and normal middle cerebral artery Doppler velocimetry to assess for anemia. Her first and second trimester serum screening studies and noninvasive prenatal testing results indicated low-risk for chromosomes 13, 18, 21, and the sex chromosomes. On diagnostic testing via amniocentesis, the fetus was found to have a normal female chromosome complement (46,XX) by Fluorescence In Situ Hybridization, karyotype, and a normal CMA. She additionally had normal carrier screening which tested for 140 single gene disorders (Counsyl. accessed April 22, 2018). Trio ES was then performed on fetal-derived amniocytes and maternal and paternal blood. After a 7-week turnaround time, at a gestational age of 30 weeks, the fetus was found to carry compound heterozygous variants in *PIEZO1*, a gene on chromosome 16 that has been previously linked to autosomal recessive lymphedema (Fotiou et al., 2015).

The fetus progressively developed worsening pleural effusions up to 2.0 cm bilaterally, scalp, facial, and abdominal wall edema to 1.4 cm, trace pericardial effusion, and marked polyhydramnios with an amniotic fluid index of 41 cm (Figure 1). The patient was subsequently admitted to the hospital at 31w4d for inpatient management and continuous fetal monitoring. She received betamethasone for fetal maturation. She developed significant maternal dyspnea and underwent two serial amnioreductions of 1.5 L and 2 L, respectively, with rapid accumulation of amniotic fluid. At 33w5d, the fetus was found to have a nonreassuring fetal heart tracing and the patient underwent urgent cesarean delivery. She delivered a female fetus weighing 2,300 g with Apgars of 1/3/3/3/4 at 1, 5, 10, 15, and 20 min, respectively. There was significant body wall edema and large bilateral pleural effusions. Bilateral chest tubes were placed; however, the neonate continued to have respiratory failure because of severely hypoplastic lungs and demised on hour 6 of life (Supporting Information Figure S2). Autopsy report confirmed congenital lymphedema Type III. The patient recovered appropriately from her cesarean delivery and was discharged home on postoperative day 3.

3 | MATERIALS AND METHODS

3.1 | Exome sequencing

Trio ES was performed at the UCLA Clinical Genomics Center. DNA was extracted from fetal amniocytes from the proband fetus affected by hydrops and from peripheral blood from unaffected parents. ES was performed using the Agilent SureSelect Clinical Research Exome XT kit and sequenced with an Illumina HiSeq 2500. A total 6,755,043,457 bases of sequence were generated and uniquely aligned to both the human reference genome and mitochondrial genome, generating a mean coverage of 84× per base within the RefSeq protein coding bases of the human genome. Approximately, 100% of the mitochondrial genome was covered to a depth of ≥4×. To identify a causative gene, a set of phenotypic keywords was generated outlining the suspected clinical features in the fetus (Lee et al., 2014). The keywords were used to create a list of 1,217 associated genes. Variant filtering was then performed following the process outline in Supporting Information Figure S3. Primer sequences were designed using Primer3 to cover the genomic regions surrounding both variants in *PIEZO1* using the transcript NM_001142864.3. (Keywords, genes, and sequences are available upon request.) DNA was extracted from formalin-fixed sections from the autopsy specimen of fetus II-B and polymerase chain reaction (PCR) and Sanger sequencing was performed on an ABI3130xl.

3.2 | Pathology

Representative sections of the organs, umbilical cord, and placenta of II-C were submitted and stained with hematoxylin and eosin (H&E). An immunohistochemical (IHC) stain was performed on histologic sections of skin and subcutaneous tissue for podoplanin, a membrane protein on lymphatic endothelium. Skin collected from other fetuses of similar gestational age were also processed and stained with H&E and podoplanin IHC. Slides were then examined by a specialist in pediatric pathology.

4 | RESULTS

4.1 | Exome and Sanger sequencing

ES identified compound heterozygous variants in the *PIEZO1* gene. This is a piezo type mechanosensitive ion channel component 1, that

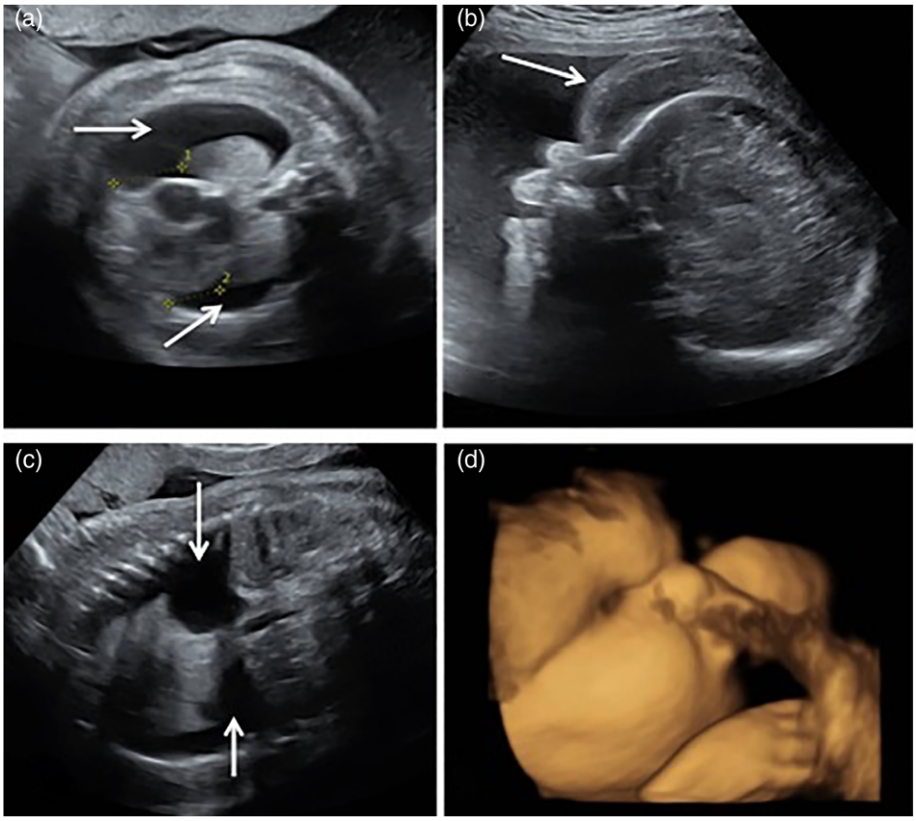

FIGURE 1 (a) Fetal ultrasound at 32w4d gestation with bilateral pleural effusions on axial view and (b) scalp edema on sagittal view. (c) Fetal ultrasound at 33w4d demonstrates bilateral pleural effusions in the sagittal view and (d) 3-dimensional ultrasound demonstrates significant facial edema [Color figure can be viewed at wileyonlinelibrary.com]

has been implicated in autosomal recessive hereditary lymphedema III (OMIM#616843; Fotiou et al., 2015) as well as autosomal dominant Dehydrated Hereditary Stomatocytosis (OMIM#194380; Zarychanski et al., 2012). Two variants in *PIEZO1* (c.3206G>A and c.6208A>C) were identified that were inherited from the father and mother, respectively. These variants are predicted to cause a nonsense (p.Trp1069*) and missense (p.Lys2070Gln) change, respectively, in the *PIEZO1* subunits. Sanger sequencing also confirmed both variants in II-B, the previous stillbirth (Supporting Information Figure S4). We performed in silico analysis for both variants using dbNSFP v2.0 (Liu, Jian, & Boerwinkle, 2013). Based on this analysis, the stop-gained/nonsense mutation was not predicted to be tolerated by any of six algorithms. For the missense variant, only one of the six algorithms predicted this mutation to be tolerated. These mutations have never been observed in gnomAD or ExAC. No other pathogenic or likely pathogenic variants in the primary gene list were identified.

4.2 | Pathology

The external examination revealed diffuse facial and corporal edema, consistent with fetal hydrops. The neonate was large for gestational age with a weight of 3,090 g (>95th percentile) with macrocephaly as a result of scalp edema (head circumference 38 cm; >95th percentile). Bilateral pleural effusions (left: 25 mL, right: 3 mL), ascites (25 mL), and a pericardial effusion (3 mL) were noted. The lungs were severely hypoplastic with a combined lung weight of 16.5 g (mean: 46.6 g with 95% range of 19.6–73.6 g) and a pulmonary to body weight ratio of 0.5 (reference for gestational age 2.59 $\pm$ 0.32). Histologic sections of the skin and subcutaneous tissue revealed an increase in the number of lymphatic channels when compared to those of unaffected fetuses of similar gestational age. The lumens of the lymphatic channels formed cord-like structures with failure of luminal canalization (highlighted by podoplanin immunohistochemistry; Supporting Information Figure S5). The placenta was also large for gestational age (647 g, >97th percentile). Histologic sections showed hydropic chorionic villi with trophoblastic basement membrane ferruginization and increased numbers of nucleated red blood cells in villus capillaries. Fetal vascular malperfusion with mural fibrin deposition and intimal fibromuscular sclerosis was also seen. The trivascular umbilical cord was hypercoiled but had no microscopic abnormalities.

5 | DISCUSSION

5.1 | Genetic causes of generalized lymphatic dysplasia

To date, there have been four genes identified to cause an autosomal recessive form of GLD with drastically varying outcomes. The three genes classically known to cause GLD are *CCBE1*, *FAT4*, and *ADAMS13* described as Hennekam lymphangiectasia-lymphedema syndrome 1 and 2 (OMIM#235510, OMIM#616006). Patients with GLD because of Hennekam syndrome have severe full body edema, dysmorphic facies, seizures, microcephaly, growth retardation, and

TABLE 1 *PIEZO1* variants reported in association with recessive generalized lymphatic dysplasia

Patient	Zygosity	Coding change (NM_001142864.3)	Protein change	Exon (51 exons)	Previously reported in
II-B, II-C	Compound heterozygous	c.3206G>A	p.Trp1069*	23	Current report
	Compound heterozygous	c.6208A>C	p.Lys2070Gln	43	Current report
GLD2 II.1, II.2*	Compound heterozygous	c.2263G>T	p.E755*	17	Fotiou et al. (2015)
	Compound heterozygous	c.6682C>T	p.Q2228X	46	
GLD1 II.2*, II.3 GLD5	Homozygous	c.4888G>T	p.E1630*	36	Fotiou et al. (2015)
Not reported	Compound heterozygous	c.6085G>C	p.G2029R	42	Lukacs et al. (2015)
	Compound heterozygous	c.3455+1G>A	p.1153Wfs21*, splice donor	24i	
GLD3 II.1*	Compound heterozygous	c.6511RG>T	p.2171F	45	Fotiou et al. (2015)
	Compound heterozygous	c.3796+1G>A	Splice donor	26.i	
GLD4 II.1, II.2, II.3	Compound heterozygous	c.7289C>T	p.2430 L	50	Fotiou et al. (2015)
	Compound heterozygous	c.1669+1G>A	27*neocodons	13i	
GLD6 II.1	Compound heterozygous	c.7366C>T	p.R2456C	51	Fotiou et al. (2015)
	Compound heterozygous, both inherited from mother	c.7374C>G	p.F2458L	51	
		c.2815C>A	p.L939M	21	

Note. Adapted from Alper (2017).

intellectual disability. Rarely, Hennekam syndrome has been reported with fetal hydrops (Bellini et al., 2003; Van Balkom et al., 2002). The fourth gene, *PIEZO1*, encodes a mechanically activated ion-channel and is important in red blood cell morphology and development of lymphatic structures. A recent review (Martin-Almedina, Mansour, & Ostergaard, 2018) discussed two distinct phenotypes caused by *PIEZO* mutations. Gain-of-function mutations in this gene are a known cause of Dehydrated Hereditary Stomatocytosis leading to hemolytic anemia (Albuisson et al., 2013; Bae, Gnanasambandam, Nicolai, Sachs, & Gottlieb, 2013). More recently, it has been discovered that loss-of-function variants in *PIEZO1* are also found to be a cause of lymphatic dysplasia (Lukacs et al., 2015). More commonly than in Hennekam syndrome, variants in *PIEZO1* appear to cause fetal onset lymphedema with fewer neonatal complications, specifically absence of seizures or intellectual disability. This report provides further evidence that the *PIEZO1* gene is a cause of autosomal recessive GLD with in utero onset.

5.2 | *PIEZO1* variants and genotype–phenotype association

PIEZO1 is a highly conserved mammalian protein that functions to regulate electrical currents using pressure sensor mechanisms at the plasma membrane (Alper, 2017). The protein forms a trimeric structure at the plasma membrane with multiple transmembrane domains allowing for mechanotransduction of force outside of the cell. The gene is widely expressed across tissue and cell types, resulting in cell-type specific phenotypes in model organisms (GTex. accessed April 22, 2018). Knockout of *PIEZO1* homologs in drosophila sensory neurons results in a lost response to noxious mechanical stimulation and decreased ability to feel pain. In mouse models, PIEZO1 is an important component of vascular formation, sensing blood flow to develop a normal vascular system. Complete or endothelial cell specific knockout of *PIEZO1* results in embryonic lethality shortly after cardiac formation and fetal heart tones are noted. Haploinsufficiency results in abnormal vasculature formation, but no other appreciable defects (Li et al. 2014).

This report contributes to the complexity of the genotype–phenotype association of the *PIEZO1* gene. In a recent review of 10 patients within 6 families, 7 of the probands were diagnosed with NIHF including one in utero demise and one neonatal demise (Fotiou et al., 2015). The remaining patients lived past 1 year of age with the oldest presently in her 30s. Those individuals that survived the neonatal period demonstrated complete resolution of the edema but with childhood recurrence of lymphedema. Rarely patients develop systemic involvement such as splenomegaly, intestinal lymphangiectasia, and gastroesophageal reflux. An additional report describes an older sibling born with severe hydrops requiring extracorporeal membrane oxygenation who developed chronic pleural effusions and persistent lymphedema but normal intelligence. The second sibling had minor lymphedema and transient pleural effusions at 3 months of age (Lukacs et al., 2015). The report of our family demonstrates a worse phenotype of the *PIEZO1* variant as illustrated by three fetal and neonatal demises because of severe lymphatic dysplasia. One question that remains is why the phenotype seen in our family was so severe, while the majority of patients reported in the literature with *PIEZO1*-associated GLD had resolution of the hydrops and survival past birth. The varying severity is highlighted by the considerable intrafamilial variation seen between siblings carrying the same *PIEZO1* variants suggesting additional genetic modifiers or possible environmental effects. Additionally, as the majority of patients thus far have been ascertained postnatally, the contribution of *PIEZO1* variants to severe NIHF in cases with in utero or neonatal demise may be underestimated. The poor outcomes seen for all three siblings in our family suggest that this might be the case. The possibility that the specific variants described here may negatively impact the prognosis also cannot be excluded.

In summary, we report novel nonsense and missense variants that have not been described in cases of *PIEZO1*-associated NIHF. Neither identified variant has been listed in the publicly available genomics databases such as ExAC/gnomAD or in affected individuals represented in Clinvar (Lek et al., 2016). To date, there are 14 mutant alleles of the *PIEZO1* gene described to cause GLD and NIHF (Alper, 2017; Table 1). Importantly, we confirmed via Sanger sequencing, the *PIEZO1* variants in the prior hydropic fetus, II-B. While an autopsy was not performed on the patient's first fetal demise, II-A, given the rarity of IUFD, it is reasonable to surmise that the etiology was identical.

5.3 | Prenatal exome sequencing

ES has been well studied in the postnatal setting specifically in autopsies and products of conception of deceased fetuses with prenatally diagnosed anomalies (Yates et al., 2017) or hydrops (Reichert, McKay, & Moldenhauer, 2016; Shehab, Tester, Ackerman, Cowchock, & Ackerman, 2017). More recently, it has been used prenatally in fetuses diagnosed with abnormalities on ultrasound with a recent review of 31 studies demonstrating a congregate diagnostic yield of ES of 25%–30% after a normal karyotype and CMA (Best et al., 2017). In one of the larger studies, (Wapner et al., 2017), among 168 fetuses with ultrasonographic abnormalities and normal karyotype and CMA, 7 had hydrops or lymphatic obstruction. Of these, ES diagnosed one with a pathologic single gene variant.

The limitations of prenatal ES include the potentially prohibitive cost, the challenges of dealing with variants of uncertain significance, and the turnaround time of 4–8 weeks that will often push women past the gestational age allowable for pregnancy interventions. Perhaps, the greatest use of obtaining a diagnosis, however, is to provide peace of mind for the patient, be able to counsel on her risk of recurrence and most importantly, provide an option for prenatal genetic diagnosis and in vitro fertilization in the future.

In summary, we report the use of prenatal ES to identify novel *PIEZO1* variants as a cause of severe in utero onset of GLD. Prediction of postnatal severity based on genotype is problematic and raises complex ethical questions about the applicability of genome-wide sequencing in the prenatal setting. Nevertheless, identification of an underlying genetic cause in families with recurrent fetal demises provides options for advanced conception using preimplantation genetic diagnosis and other reproductive options in the future.

CONFLICT OF INTEREST

None.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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