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CLINICAL REPORT

A homozygous variant in growth and differentiation factor 2 *(GDF2)* may cause lymphatic dysplasia with hydrothorax and nonimmune hydrops fetalis

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Abstract

The etiology of nonimmune hydrops fetalis is extensive and includes genetic disorders. We describe a term-born female neonate with late onset extensive nonimmune hydrops, that is, polyhydramnios, edema, and congenital bilateral chylothorax. This newborn was successfully treated with repetitive thoracocentesis, total parenteral feeding, octreotide intravenously and finally surgical pleurodesis and corticosteroids. A genetic cause seemed plausible as the maternal history revealed a fatal nonimmune hydrops fetalis. A homozygous truncating variant in *GDF2* (c.451C>T, p.(Arg151*)) was detected with exome sequencing. Genetic analysis of tissue obtained from the deceased fetal sibling revealed the same homozygous variant. The parents and two healthy siblings were heterozygous for the *GDF2* variant. Skin and lung biopsies in the index patient, as well as the revised lung biopsy of the deceased fetal sibling, showed lymphatic dysplasia and lymphangiectasia. To the best of our knowledge, this is the first report of an association between a homozygous variant in *GDF2* with lymphatic dysplasia, hydrothorax and nonimmune hydrops fetalis.

KEYWORDS

BMP9, GDF2, hereditary hemorrhagic telangiectasia, lymphatic dysplasia, nonimmune hydrops fetalis, pulmonary arterial hypertension

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1 | INTRODUCTION

Nonimmune hydrops fetalis is a condition characterized by excessive accumulation of fluid in at least two fetal extravascular compartments or body cavities, including ascites, pericardial effusion, pleural effusion and skin edema. Placental thickening and polyhydramnios are other frequent findings. The majority of cases of hydrops fetalis is classified as nonimmune hydrops fetalis (NIHF), that refers to hydrops due to other causes than red cell alloimmunization (Bellini et al., 2015; Norton, Chauhan, & Dashe, 2015). NIHF has a reported prevalence of 1:1700 to 1:3000 pregnancies (Norton et al., 2015) and is associated with a high mortality rate with an overall mortality up to 45% at 1 year (Fukushima et al., 2011; Nassr et al., 2018; Steurer et al., 2017). Identification of the underlying cause may guide perinatal management of contemporary cases and genetic counseling in future pregnancies (Mardy, Chetty, Norton, & Sparks, 2019; Sparks et al., 2019). NIHF has a wide array of underlying etiologies.

A recent systematic review (Bellini et al., 2015) lists 14 different etiological-categories and their relative frequencies. Cardiovascular anomalies are the most frequent cause of NIHF (≈20%). Genetic disorders may also lead to NIHF and include a wide spectrum of diseases, including chromosomal aneuplodies (trisomies, monosomy X, triploidy), hematological diseases (α-thalassemia), monogenic syndromes (RASopathies, Kabuki syndrome), inborn errors of metabolism (lysosomal storage disease) and lymphatic dysplasia (Bellini et al., 2015; Mardy et al., 2019; McPherson, 2019; Moreno et al., 2013; Quinlan-Jones et al., 2019; Weissbach et al., 2019). Few familial cases of nonimmune hydrops fetalis have been described and in some of the cases with lymphatic dysplasia and/or lymphangiectasia bi-allelic variants in *CCBE1*, *ADAMTS3*, *FAT4*, *CALCRL*, and *PIEZO1* have been reported (Alders et al., 2009; Alders et al., 2014; Brouillard et al., 2017; Datkhaeva et al., 2018; Delabaere et al., 2008; Fotiou et al., 2015; Jacquemont, Barbarot, Boceno, Stalder, & David, 2000; Mackie et al., 2018; Njolstad, Reigstad, Westby, & Espeland, 1998; Stevenson, Pysher, Ward, & Carey, 2006; Wieacker, Muschke, Pollak, & Muller, 2005). However, even with conventional and state-of-the-art diagnostics like exome sequencing an underlying genetic defect remains undetermined in the majority of infants with hydrops (Lord et al., 2019; Yates et al., 2017). A genetic diagnosis for NIHF may affect counseling during the pregnancy, neonatal management as well as genetic counseling in future pregnancies including the possibility of preimplantation diagnostics.

In this report, we describe the familial occurrence of late onset nonimmune hydrops fetalis in two siblings in which we identified a homozygous truncating variant in *GDF2*. To the best of our knowledge, an association between *GDF2* with lymphatic dysplasia, hydrothorax, and NIHF has not been described previously. We propose an etiologic role of this gene in lymphatic dysplasia and concurrent NIHF.

2 | PATIENTS AND METHODS

2.1 | Patients

A family with two siblings with late onset (McPherson, 2019) NIHF and two healthy siblings is reported. The parents of the index patient signed a written informed consent for rapid diagnostic exome sequencing (trio analysis of the proband, mother and father), consented with targeted variant analysis of the three siblings and signed consent for publication.

2.2 | Immunohistochemistry

Tissues were fixed in neutral buffered formalin and embedded in paraffin. Immunohistochemistry was performed on fresh 3 μm sections on a Ventana Benchmark Ultra autostainer (Ventana Medical Systems / Roche-diagnostics, Tucson, AZ). For immunohistochemical demonstration of podoplanin (PDPN) the D2-40 mouse monoclonal antibody was used (Roche Diagnostics, Catalogue Number 760-4395) in accordance with the manufacturer's instructions.

2.3 | Molecular studies

Exome sequencing and variant calling were performed as previously described (Herkert et al., 2018). In brief, the exome was captured with the Agilent SureSelect XT Human All Exon V6 kit (Agilent, Santa Clara, CA) and exome libraries were sequenced on a NextSeq500 (Illumina, San Diego, CA) with 2x 150 bp paired-end reads at an average coverage of 100x and with >90% of the exome covered >20x. Sequence reads were aligned to the human reference genome (UCSC version GRCh37/hg19) with the Burrows-Wheeler Aligner version 0.7.5a. Sambamba was used to process the aligned reads, after which we applied Genome Analysis Tool Kit (GATK) duplicate removal and performed SNP and INDEL discovery and genotyping using standard hard filtering parameters according to GATK Best Practices recommendations. Sequence variants were filtered with Cartagenia Next-Generation Sequencing–Bench Laboratory software (Agilent, Santa Clara, CA) by using an automated filtering tree. Variant classification was done according to the ACMG-guidelines (Richards et al., 2015). For data-analysis an updated version of a previously published virtual genepanel (van Diemen et al., 2017) containing approximately 3,850 genes at the time of analysis was used. In brief, this virtual gene panel contains monogenic diseases listed in the clinical genomic database (CGD) with the exception that genes associated with late-onset diseases were removed (van Diemen et al., 2017). In addition, individual genes from a standard, clinical exome-capturing panel (SureSelect Inherited Disease; Agilent, Santa Clara, CA) but not included in the CGD were added.

2.4 | Review of *GDF2* variants

We mined the published literature for patients with a germline variant in *GDF2* and summarized their genotypes and associated phenotypes (Abou Hassan et al., 2018; Eyries et al., 2019; Graf et al., 2018; Hernandez et al., 2015; Hodgson et al., 2019; G. Wang et al., 2016; X.-J. Wang et al., 2019; Wooderchak-Donahue et al., 2013; Yang et al., 2018; Zhu et al., 2019) including the present study.

3 | CLINICAL REPORT

3.1 | Case report

3.1.1 | Prenatal history

A female neonate was born via an emergency cesarean section at term to a multigravida (G5) with two healthy children. The maternal obstetric history revealed a spontaneous abortion at ~10 weeks and an intrauterine fetal death at 33 weeks of gestation. The intrauterine fetal death was due to nonimmune hydrops fetalis with hydrothorax and amniocentesis revealed a mosaic trisomy 20 by both conventional karyotyping (47,XX,+20[7]/46,XX[4]) and array analysis.The current pregnancy was again complicated by late onset NIHF with polyhydramnios and a right-sided hydrothorax first detected at 34 + 6 weeks of gestation. Prenatal evaluation showed no evidence of immune hydrops, congenital infections, or cardiac abnormalities.

3.1.2 | Post partum diagnostic evaluation and management

After birth extensive subcutaneous edema, especially in the head and neck region, without other dysmorphic features, was observed in this newborn. Apgar scores were 2, 7, and 8 after 1, 5, and 10 min, respectively. Respiratory failure developed immediately after birth requiring mechanical ventilation. A chest X-ray showed bilateral pleural effusions. Chest tubes were placed; the biochemical analyses of the fluid was consistent with chyle. Additional treatment consisted of nil per os and total parenteral nutrition. Chylous volumes averaged upto 900 ml per day, and therefore intravenous infusion of Octreotide was started. Initially there was a gradual decrease in chylous volumes, so after several weeks chest tubes could be removed and Octreotide infusion could be discontinued. Unfortunately, the production of chyle increased after a medium-chain triglyceride diet was started. Enteral feeding was discontinued and Octreotide was restarted. In the meantime, a high-resolution computed tomography (HRCT) of the chest was suggestive for interstitial lung disease with diffuse ground glass opacities, thickened inter- and intralobar septa and prominent pulmonary vessels. These findings suggested an obstructive disorder of the lymphatic outflow trajectory.

3.1.3 | Surgical management and follow-up

Surgical treatment was deemed necessary after weeks of mechanical ventilation and failure of conservative management. A video-assisted thoracic surgery (VATS) on the left side was performed with a pleurectomy and pleural drainage. Due to the presence of adhesions the right-sided VATS was converted to a thoracotomy and subsequently chemical pleurodesis was performed. A lung and skin biopsy were taken in order to provide histopathological support for the presumed diagnosis. Lung histology showed interstitial emphysema, and some lymphangiectasia, and interstitial pathology compatible with pulmonary interstitial glycogenosis (Figure 1b). Skin histology showed features of lymphatic vascular malformations (Figure 1c). Given the severity of the disease and uncertainty about the contributing role of the pulmonary interstitial glycogenosis, methylprednisolone therapy was given for 3 days. Clinical improvement occurred followed by an uneventful neonatal course. Currently, at the age of 1 year, she is doing fine on a normal diet without respiratory symptoms, without hepatosplenomegaly and without signs of peripheral lymphedema. She has a normal mental and psychomotor development.

3.1.4 | Molecular studies

A SNP-array showed no relevant chromosomal numerical abnormalities and no evidence for a (large) deletion of the *GBA* locus. In addition several large (>9 Mb) regions of homozygosity were identified (including the *GDF2* region). Targeted exome sequencing was performed on DNA from the proband and her parents. A homozygous truncating variant in *GDF2* (c.451C > T, p.(Arg151*)), and two heterozygous variants (in *WNT10A* and *GBA)* were identified. Both parents were heterozygous for the *GDF2* variant. The *WNT10A* was paternally inherited and interpreted as unrelated to the phenotype. The heterozygous *GBA* variant was maternally inherited and no second variant was found. The SNP-array was re-analyzed for the *GBA* locus and no deletion was detected. Although small deletions below the SNP-array resolution cannot be fully excluded these seem very rare in *GBA* (Pastores & Hughes, 1993). Moreover, clinically there were no signs of Gaucher's disease (normal beta-glucosidase/glucocerebrosidase activity and no increased plasma chitotriosidase activity). Therefore autosomal recessive Gaucher's disease was excluded as a cause of the fetal hydrops. Subsequent analysis of the *GDF2* variant by targeted sequencing identified homozygosity of c.451C > T / p.(Arg151*) in the affected stillbirth and heterozygosity in her unaffected siblings (Figure S4). A submission of the variant in GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2015) did not result in any matches for homozygous or compound heterozygous variants (match status last checked 04.05.2020).

4 | DISCUSSION

4.1 | General discussion

We report a family with two siblings affected by fetal hydrops carrying a homozygous truncating variant in *GDF2*. *GDF2* encodes the

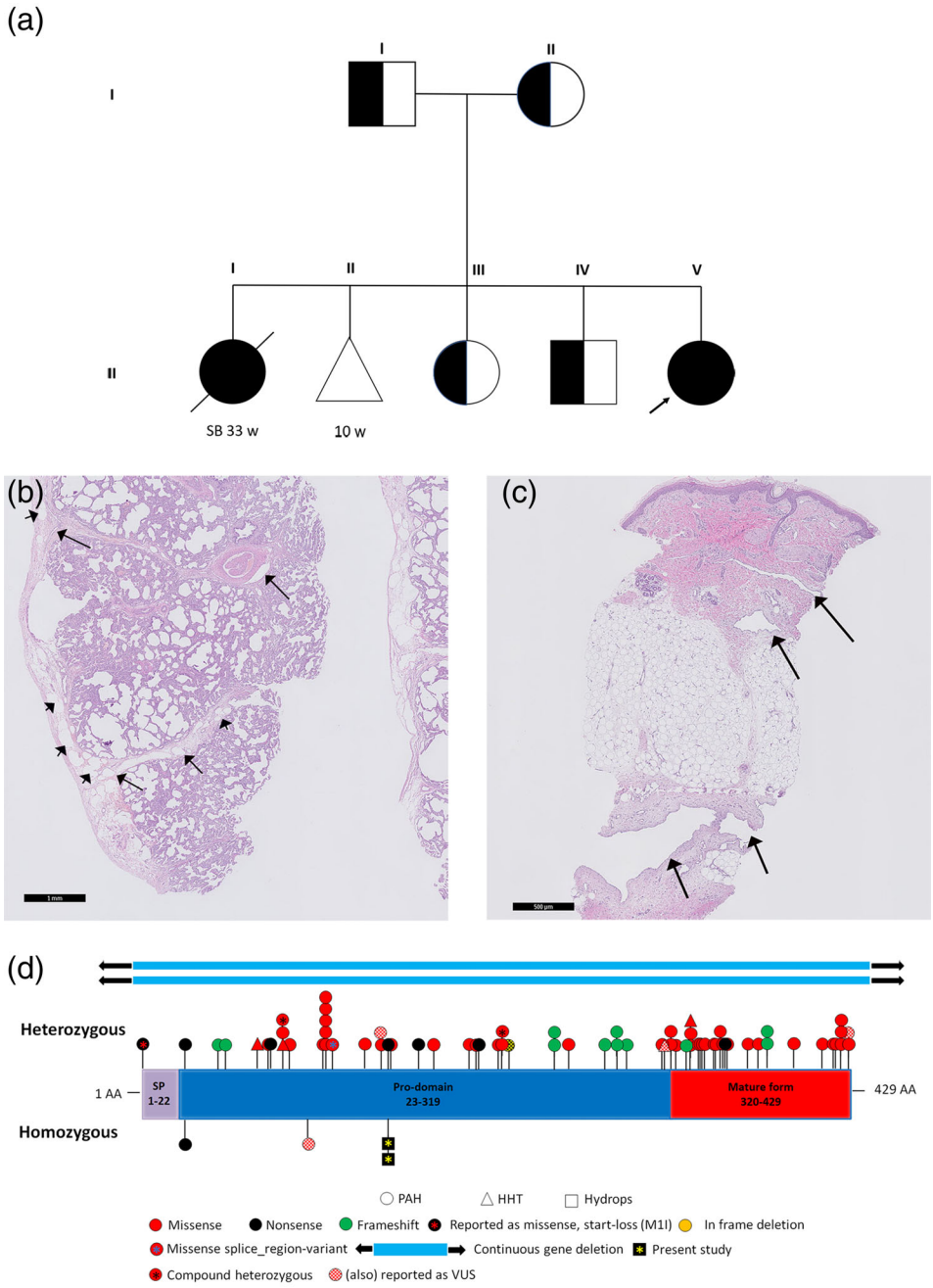

FIGURE 1 Pedigree, histopathological evaluation and genotype—phenotype characteristics of (published) *GDF2* variants. (a) pedigree of the family with unaffected heterozygous depicted by half-filled symbols and affected homozygous individuals by filled symbols. The index patients is indicated by an arrow ↗. (b,c) representative histopathological analysis of lung and skin biopsies of the index patient (b) lung biopsy (×40) and (c) skin biopsy. (B) lung biopsy (bar = 1 mm) showing lymphangiectasis (long arrows) as well as interstitial emphysema (short arrows) and (c) skin biopsy (bar = 0,5 mm) showing lymphangiectasis in upper dermis as well as subcutis (arrows). The lymphatic origin was confirmed with D2-40 (PDPN) staining (data not shown). (d) schematic representation of published *GDF2*-variants. Protein domains according to Wang et al. (X.-J. Wang et al., 2019) and UniProt, https://www.uniprot.org/uniprot/Q9UK05. Lay-out adapted from Wagener et al. (Wagener et al., 2015) SP indicates signal peptide, AA amino acid. Splice-site variants are mapped against the amino-acid position corresponding to the position of the nucleotide change (Skoric-Milosavljevic et al., 2019). Different variants at the nucleotide level may lead to different amino-acid substitutions at the same amino-acid position. Variants reported in control cohorts as well as those classified as (likely) benign in the reviewed papers were not included in the figure

circulating bone morphogenetic protein 9 (BMP9), a protein that plays a role in angiogenesis. BMP9 is a ligand that binds with high affinity to activin receptor-like kinase 1 (ALK1) and Endoglin, members of the transforming growth factor-β family (TGFβ) and is expressed in endothelial cells (Kienast et al., 2016; Lawera et al., 2019; Saito et al., 2017; Townson et al., 2012). The BMP9/ALK1/Endoglin pathway is involved in the regulation of angiogenesis by inhibition of endothelial cell proliferation and migration (David, Feige, & Bailly, 2009). In addition to its role in angiogenesis TGFβ signaling is also involved in lymphatic development (James, Nalbandian, & Mukouyama, 2013; Oka et al., 2008). Indeed, analysis of the STRING database (https://string-db.org/) shows an enrichment of protein–protein interactions between GDF2 and other proteins encoded by germline predisposition genes involved in pulmonary arterial hypertension (PAH) and hereditary hemorrhagic telangiectasia (HHT) (Figure S1) as well as overlap between the genotypes and phenotypes of PAH, HHT, and hydrops (Figure S2). Variants in *GDF2* (almost exclusively heterozygous variants) have emerged as disease causing in HHT (MIM #615506) and PAH (Figure 1d) (Abou Hassan et al., 2018; Eyries et al., 2019; Graf et al., 2018; Hernandez et al., 2015; Hodgson et al., 2019; G. Wang et al., 2016; X.-J. Wang et al., 2019; Wooderchak-Donahue et al., 2013; Zhu et al., 2019). These variants are predominantly missense and protein truncating variants (nonsense and frameshift) and loss-of-function has been proposed as the pathogenic mechanism (Hodgson et al., 2019; Southgate, Machado, Graf, & Morrell, 2019). Although no clear hotspots exist, ~60% of the variants

affect the relatively small mature domain (109 AA) either directly by missense or by protein truncating variants (Figure S3). The majority of the protein truncating variants (including the Arg151* in our patients) will likely not be subjected to nonsense mediated mRNA decay (NMD) as the premature termination codons (PTC) are located in the terminal exon of the gene (exon 2) (Kurosaki, Popp, & Maquat, 2019; Lykke-Andersen & Jensen, 2015). As no functional studies were conducted it remains unclear if indeed a truncated protein is formed and whether there is any residual protein activity.

4.2 | The pulmonary arterial hypertension (PAH) phenotype

Homozygous and compound heterozygous variants in *GDF2* have been very rarely reported previously. Wang et al. (G. Wang et al., 2016) reported a child with PAH carrying a homozygous truncating variant (c.76C > T, p.[Gln26*]). In this case the early onset and severity of PAH is most likely due to the homozygosity of the variant. Incomplete penetrance of the heterozygous variant may account for the negative family history of PAH in both the parental and maternal family. Incomplete penetrance is supported by the finding that most of the reported patients with PAH and *GDF2* variants represent sporadic/idiopathic cases without a family history of PAH (Eyries et al., 2019; Graf et al., 2018; Hodgson et al., 2019; G. Wang et al., 2016; Zhu et al., 2019). Nonpenetrance of the heterozygous variant may also be the case in our family. In the series published by Abou Hassan et al. (Abou Hassan et al., 2018) one patient with PAH was compound heterozygous for two variants (c.254C>T, p.(Pro85Leu)/c.652G>A, p.(Asp218Asn)). However, as the unaffected father of this patient carried the p.(Asp218Asn) variant in homozygous state and as this variant is frequently detected (in homozygous state) in non-PAH conditions (Hodgson et al., 2019) and has been classified as benign based on functional studies by Hodgson et al. it is unclear whether this compound heterozygous variant is comparable with other reported bi-allelic variants. Finally, a homozygous variant of undetermined clinical significance (VUS) was reported in a PAH patient by Yang et al.(Yang et al., 2018). However, the phenotype of hydrops and hydrothorax/chylothorax as seen in our family has not been reported yet. The lymphangiectasia observed in our patients are unlikely to be a manifestation of PAH as in PAH only very small angiectasies can be seen and only as component of the characteristic plexiform lesions as seen in advanced stages. In PAH teleangiectasies/lymphangiectasies are not part of the histopathological spectrum.

4.3 | The lymphatic dysplasia and hydrops phenotype

Several observations support an etiologic role for the homozygous *GDF2* variant in this NIHF phenotype. First, the two affected patients were homozygous for the c.451C > T, p.(Arg151*) while the unaffected siblings and parents were heterozygous. Second, the skin- and lung biopsies in the index patient and affected sibling showed lymphatic dysplasia and lymphangiectasia (Figure 1b,c). The lymphangiectasia in the affected sibling was identified only after histopathological and immunohistochemical re-evaluation confirming the observation that lymphatic dysplasia is a likely underestimated etiology in the evaluation of NIHF when relying on histopathological evaluation only (Bellini et al., 2010). The third reason to suggest an etiological role for *GDF2* variants in NIHF is supplied by animal studies: *Bmp9* knock-out (KO) mice show several defects in lymphatic development and function (Levet et al., 2013; Yoshimatsu et al., 2013). As noted before, BMP9 is the ligand for the ALK1 receptor and blockade of ALK1 signaling also results in defects in lymphatic development (Niessen, Zhang, Ridgway, Chen, & Yan, 2010). Fourth, with SNP-array analysis and massive parallel sequencing other major genetic causes of NIHF were excluded. This included hematological disease, chromosomal aneuploidies, and variants in genes known to cause lymphatic dysplasia including RASopathies, (generalized) lymphedema, or inborn errors of metabolism (Bellini et al., 2015; Hakami, Dillon, Lebo, & Mason-Suares, 2016; Houweling et al., 2010; Johnston et al., 2018; Joyce et al., 2016; Mardy et al., 2019; Martin-Almedina et al., 2016; Mason-Suares et al., 2017; Meng et al., 2019; Moreno et al., 2013; Pagnamenta et al., 2019; Quinlan-Jones et al., 2019; Stuurman et al., 2019; Sudrie-Arnaud et al., 2018; Weissbach et al., 2019; Yates et al., 2017). Finally, no other causes for the NIHF, such as intra-uterine infections and cardiac abnormalities, were present. The mosaic trisomy 20 observed in cultured amnion cells from the deceased fetal sibling is unlikely to have played a role as no single case of (mosaic) trisomy 20 was identified among 1,004 cases (including 199 with chromosomal abnormalities) with NIHF in the series published by Meng et al. (Meng et al., 2019) and for reasons outlined below (see "the pathophysiological mechanism").

4.4 | The pathophysiological mechanism

Despite this specific genetic diagnosis of a homozygous variant in *GDF2*, the exact pathophysiology in these NIHF cases remains not fully elucidated for at least two reasons: First, the histological findings in the index patient were characteristic for pulmonary interstitial glycogenosis (PIG, a rare pediatric lung disease characterized by accumulation of cytoplasmic glycogen in mesenchymal cells in the alveolar interstitium, with unknown underlying etiology and clinical significance). This glycogenosis is associated with a variety of neonatal pulmonary and cardiovascular disorders and may appear to be transient with usually a favorable prognosis (Canakis, Cutz, Manson, & O'Brodovich, 2002; Cutz, Chami, Dell, Langer, & Manson, 2017; Seidl et al., 2018). The combination of PIG, pulmonary lymphangiectasia, and pleural effusion has been described previously (Cutz et al., 2017; Deutsch & Young, 2016) and supports the finding that PIG is associated with pulmonary disorders, including an abnormal lung development such as pulmonary lymphangiectasia. We presume that PIG is not responsible for the main symptoms in our case, but it is tempting to speculate that PIG is part of the phenotype with hydrops and

chylothorax or even is a consequence of impaired differentiation or maturation of the lung due to the pulmonary abnormalities. Second, the multi-treatment-strategy for our patient makes it impossible to discriminate between specific treatment effects and their role in clinical recovery. Repeating a lung HRCT and biopsy might potentially help to unravel this diagnostic issues, but is obviously potentially harmful and therefore not performed. Despite both affected siblings had similar prenatal ultrasonographic findings with hydrops and hydrothorax and both were homozygous for the *GDF2* variant the outcome was fatal in the prior pregnancy were also a mosaic trisomy 20 was detected. Mosaic trisomy 20 is a frequent finding in invasive prenatal diagnostics and (likely) in the majority of cases of extraembryonic origin (R Wallerstein et al., 2000). It is not clearly associated with a specific phenotype (Bianca et al., 2008; Robinson et al., 2005; Willis, Bird, Dell'Aquilla, & Jones, 2008) but a poor outcome has been reported in individual cases (with a high-level mosaicism for trisomy 20) (Robinson et al., 2005; Robert Wallerstein et al., 2015). So the mosaic trisomy 20 might have contributed to the fatal outcome. However, in our opinion a full relation between the mosaic trisomy 20 and the hydrops phenotype seems unlikely (Robinson et al., 2005; Robert Wallerstein et al., 2015).

4.5 | Concluding remarks

In conclusion we report on a family with fatal as well as nonfatal NIHF in two siblings and propose, based on molecular evaluation, clinical- and histopathological analyses and literature review a critical role for *GDF2* in the observed phenotype. Although some uncertainty remains about the relation between this gene and the presenting symptoms, it is important to consider this specific genetic cause in case of unexplained NIHF. Hopefully other cases will be recognized after this report to confirm the causal relation. The identification of the underlying genetic defect not only provides important information for genetic counseling in future pregnancies (with a recurrence risk of 25% in the present family) but it also provides an option for preimplantation diagnostics. In addition, this report may affect perinatal counseling and management for further families. In particular because we reported not only a fatal case, but also a favorable outcome after intensive treatment of an infant with NIHF.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Designed the research: Sietse M. Aukema, Gerdien A. ten Brinke, Christian V. Hulzebos, Wilhelmina S. Kerstjens-Frederikse. Contributed clinical or pathological data: Wim Timens, Yvonne J. Vos, Ryan E. Accord, Karianne E. Kraft, Michiel J. Santing, Leonard P. Morssink, Esther Streefland, Cleo C. van Diemen, Elianne JLE Vrijlandt. Manuscript writing: Sietse M. Aukema, Gerdien A. ten Brinke, Christian V. Hulzebos, Wilhelmina S. Kerstjens-Frederikse. Revision and approval of the manuscript: all authors.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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