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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 fetalis, 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
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 aneuploidies (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; 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, CALCR, and PIEZO1 have been reported (Alders et al., 2009; Alders et al., 2014; Bourillou et al., 2017; Datkhaeva et al., 2018; Delabaere et al., 2008; Fotiou et al., 2015; Jacquemont, Barbilot, 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 mono- genic 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.
We report a family with two siblings affected by fetal hydrops carrying a homozygous truncating variant in GDF2. GDF2 encodes the
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 (TGFβ) family 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
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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 etiologic 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”).

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
chylorothorax 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, Dall’Aquila, & 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


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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