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Research Article

PRENATAL DIAGNOSIS OF A HOMOZYGOUS VARIANT OF *SUMF1* IN A FETUS WITH HYDROPS FETALIS USING CHORIONIC VILLUS SAMPLING

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ABSTRACT

Nonimmune hydrops fetalis (NIHF) is a severe prenatal condition with a broad etiological spectrum, including chromosomal abnormalities, structural malformations, congenital infections, and inherited metabolic disorders. Early identification of the underlying cause is essential for accurate prognosis, clinical decision-making, and genetic counseling. Here, we report the prenatal diagnosis of a fetus presenting with early-onset NIHF caused by a homozygous missense variant in the *SUMF1* gene, detected through first-trimester chorionic villus sampling (CVS). A 27-year-old gravida 5 woman with a history of two previous pregnancies affected by unexplained hydrops fetalis was referred at 12 weeks and 3 days of gestation. Ultrasound examination revealed markedly increased nuchal translucency, generalized hydrops fetalis, and absence of the nasal bone. Given the recurrent phenotype and early gestational presentation, invasive prenatal diagnosis was performed using CVS. Clinical exome sequencing identified a homozygous *SUMF1* variant (NM_182760.4: c.538T>A, p.Trp180Arg), which is absent from population databases and has been reported only once in ClinVar as a variant of uncertain significance. Subsequent Sanger sequencing confirmed heterozygous carrier status in both parents and the unaffected sibling, consistent with autosomal recessive inheritance. *SUMF1* encodes the sulfatase-modifying factor 1, a critical enzyme required for post-translational activation of all sulfatases. Biallelic pathogenic variants in *SUMF1* cause multiple sulfatase deficiency, an ultra-rare lysosomal storage disorder that may present prenatally with hydrops fetalis. The severe and recurrent fetal phenotype observed in this family strongly supports the pathogenic relevance of the

identified variant. This case highlights the diagnostic value of early invasive prenatal testing, particularly CVS combined with next-generation sequencing, in pregnancies complicated by early-onset or recurrent NIHF. Early molecular diagnosis enables timely counseling, informed reproductive decision-making, and appropriate planning for future pregnancies.

Keywords: Chorionic villus sampling, exome sequencing, nonimmune hydrops fetalis, multiple sulfatase deficiency, prenatal diagnosis, *SUMF1* gene.

INTRODUCTION

Nonimmune hydrops fetalis (NIHF) is a serious fetal condition characterized by abnormal fluid accumulation in two or more fetal compartments, such as the abdominal cavity, pleural space, or subcutaneous tissue. It can result from a wide range of underlying etiologies, including cardiovascular abnormalities, chromosomal abnormalities, congenital infections, metabolic disorders, and hematologic conditions (Bellini and Hennekam, 2012). Early and accurate diagnosis is crucial for appropriate management and counseling.

Sulfatase-modifying factor 1 (SUMF1) is an essential enzyme involved in the post-translational modification and activation of sulfatases, a family of enzymes responsible for the degradation of sulfated biomolecules (Fraldi *et al.*, 2007). Biallelic pathogenic variants in the *SUMF1* gene cause multiple sulfatase deficiency (MSD, MIM#272200), a rare autosomal recessive disorder characterized by the combined deficiency of all sulfatase activities (Cosma *et al.*, 2003). MSD is severe; patients with MSD present with specific clinical findings include psychomotor retardation and neurological deterioration as well as dysostosis multiplex, organomegaly, cardiac valve disease, corneal clouding, retinopathy with vision loss, hearing loss, recurrent infections, gingival hypertrophy, joint stiffness, carpal tunnel syndrome, and neuropathy. Most individuals with MSD die before the age of 10 although several individuals have been reported to have lived into the second and third decades of life. Approximately 50 mutations of *SUMF1* have been reported (Eto *et al.*, 1987; Sabourdy *et al.*, 2015). Prenatal diagnosis of MSD and other underlying causes of NIHF is challenging but important for informed decision-making, potential interventions and counseling for future pregnancies.

Chorionic villus sampling (CVS) is a well-established invasive prenatal diagnostic procedure performed as early as the 10th week of gestation that allows for genetic testing and analysis of fetal cells obtained from the placenta. CVS is the earliest diagnostic approach, suitable for application in fetuses presenting with anomalies as early as the first trimester. It is specifically recommended for pregnancies identified as having a heightened risk of numerical and structural chromosomal abnormalities, or monogenic diseases. These risk factors are typically ascertained through antenatal screening methods such as maternal blood tests, morphological ultrasound examinations, or familial histories displaying instances of chromosomal or genetic irregularities (Practice Bulletin No. 162 Summary: Prenatal Diagnostic Testing for Genetic Disorders, 2016; Wapner, 1997). Furthermore, it is essential to exercise prudence when considering CVS procedures in patients undergoing anticoagulant therapy or those afflicted with coagulation disorders. Caution should also be exercised in cases

where patients manifest signs of infection or exhibit Rh blood group incompatibility between the mother and the fetus. Pregnant individuals carrying bloodborne infectious diseases such as HIV, hepatitis viruses with high antigen loads, or syphilis should be educated about the potential transmission risks to the fetus. The CVS procedure is contraindicated in instances where the cervical length falls below 25.0 mm, particularly in cases of threatened miscarriage. The risks associated with CVS are similar to those seen with amniocentesis and include miscarriage, bleeding, infection, rupture of the membranes, and uncertain results. Some studies evaluating the complications of transabdominal CVS have indicated a miscarriage rate of 2-3% in the entire pregnancy (Mujezinovic and Alfirevic, 2007), which is a 0.2% increase compared to pregnancies without the procedure, and this difference is not statistically significant ($p = 0.7$) (Martins *et al.*, 2020).

Herein, we report the prenatal diagnosis of a homozygous missense variant in *SUMF1* in a fetus with NIHF using CVS, in which the mother had a history of two prior pregnancies affected by hydrops fetalis without a clear genetic diagnosis.

MATERIALS AND METHODS

Study subject and clinical presentation

A 27-year-old woman, gravida 5, para 3, aborta 2, living children 1 (PARA 1221), with a history of two prior pregnancies terminated due to hydrops fetalis of undetermined etiology. In 2016, at 23 weeks gestation, one fetus presented with hydrops fetalis, and amniocentesis revealed a normal karyotype. In 2020, at 22 weeks, one fetus presented with hydrops fetalis and interventricular communication, but no prenatal diagnosis was performed.

This gestational age was 12 weeks and 3 days. Ultrasound examination demonstrated an increased nuchal translucency of 6.9 mm, hydrops fetalis, and absence of the nasal bone. The placenta was anteriorly located, and the cervical length measured 36.5 mm. We recognized that the fetus exhibited a recurrence of hydrops fetalis similar to the two previous affected pregnancies, thus warranting prenatal diagnostic evaluation via CVS. Basic laboratory tests did not reveal any abnormalities, and the pregnant woman tested negative for HIV, HBV, and *Treponema pallidum*. She has been deemed eligible for CVS. The pregnant woman had been provided with an explanation of the risks associated with the procedure, as well as information about the genetic tests that will be conducted on the chorionic villi sample.

Chorionic villus sampling procedure and sample collection

The pregnant woman had undergone CVS. An 18-gauge biopsy needle was introduced into the chorionic villi under continuous ultrasound guidance. A 20cc syringe was attached to the needle, creating negative pressure, and the needle was maneuvered up and down through the chorionic villi to collect the sample. The sample was transferred to a sample container containing 0.9% NaCl solution and re-evaluated for the collected specimen.

Genetic analysis

The chorionic villus sample was obtained and sent to the Center of Clinical Genetics of Hanoi Medical University Hospital for genetic analysis. The chorionic villi was processed under aseptic conditions, cultured

to establish a fetal cell line, and maternal cell contamination was removed. Subsequently, DNA was extracted from the chorionic villus cells and underwent next-generation sequencing (NGS) using a clinical exome panel targeting 4,503 disease-associated genes (CES – Clinical Exome Sequencing).

Structural modeling and protein sequence analysis

Structural modeling was performed to illustrate the three-dimensional conformation of the protein of interest. The reference amino acid sequence was retrieved from the UniProt database. An in silico amino acid substitution was introduced based on the reference sequence. Three-dimensional structural modeling and visualization were conducted using Swiss-PdbViewer (DeepView) version 4.1.

RESULTS

The results revealed the presence of a homozygous missense variant in the *SUMF1* gene (NM_182760.4: c.538T>A, p.Trp180Arg) which was classified as a Variant of Uncertain Significance according to ACMG guidelines (PP3 + PM5 + PM2) (Richards *et al.*, 2015). This variant was previously reported once in ClinVar and was classified as a Variant of Uncertain Significance. This *SUMF1* variant is associated with MSD, a disorder that can manifest with hydrops fetalis prenatally (Schlotawa *et al.*, 2019; Whybra *et al.*, 2012), concordant with the clinical presentation observed in this fetus. The current case demonstrates significant phenotypic overlap with the MSD case reported from Schlotawa *et al.*, particularly regarding hydrops fetalis and the potential for a poor prognosis due to a *SUMF1* variant. Compared to Whybra *et al.*, the current case exhibits a more severe phenotype, lacking the transient nature of hydrops and presenting earlier (week 12 vs. weeks 22–30). Differences in onset timing and phenotypic severity may relate to the nature of the genetic variant (missense vs. stop mutation) and diagnostic timing (prenatal vs. postnatal). These findings emphasize the importance of early prenatal diagnosis via CVS and the need to consider MSD in the differential diagnosis of NIHF, particularly in families with a history of recurrent hydrops fetalis.

We also performed Sanger sequencing on blood samples from the mother, father, and firstborn child of this parent, which revealed that each individual was heterozygous for the *SUMF1* variant (Figures 1A-C), consistent with an autosomal recessive inheritance pattern. Sanger sequencing on the chorionic villus sample also revealed the homozygous for the *SUMF1* variant (Figure 1D).

The pregnancy was terminated due to the severe fetal condition and genetic abnormality. Pregnant counseling was offered for subsequent pregnancies, including options to avoid having a fetus with the disease, such as preimplantation genetic diagnosis or prenatal diagnosis by CVS or amniocentesis.

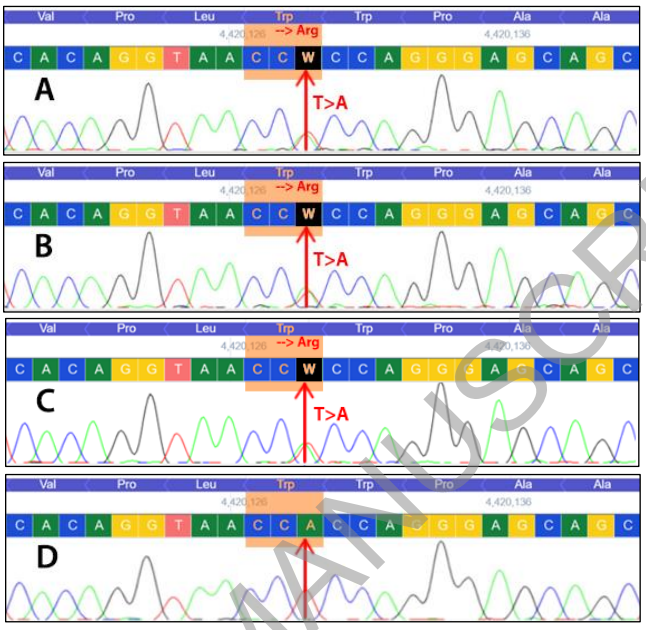

Figure 1. Sanger sequencing chromatogram (DNA reverse complement strand) of the heterozygous variant of *SUMF1* (NM_182760.4): c.538T>A in the mother (A), the father (B), the firstborn child of this parent (C), and the homozygous variant of *SUMF1*: c.538T>A in chorionic villus of the fetus (D). The RED arrow indicates the position of nucleotide substitution; the ORANGE highlighted region marks the corresponding amino acid alteration site.

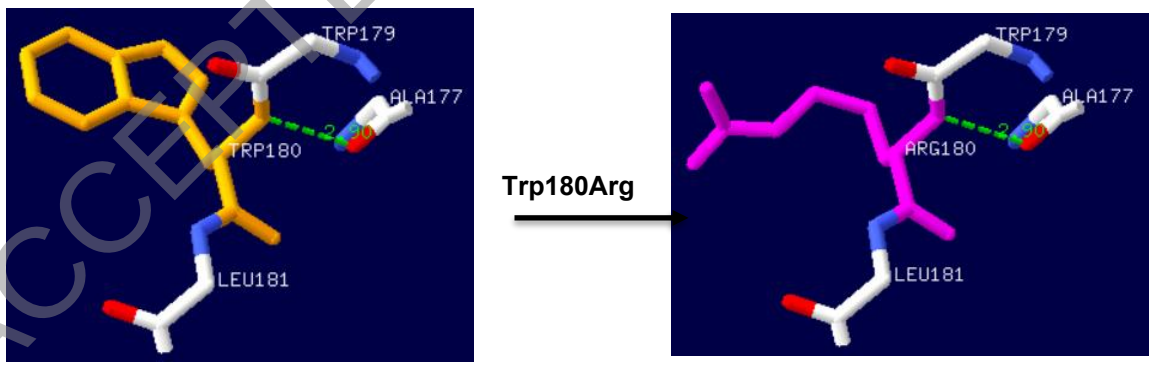

Figure 2. Structural illustration of the p.Trp180Arg substitution in the SUMF1 protein (UniProt ID: Q8NBK3). The left panel shows the wild-type tryptophan residue at position 180 (Trp180), while the right panel displays the mutant arginine residue (Arg180) at the same position. Replacement of the hydrophobic tryptophan with a positively charged arginine is predicted to disrupt local protein folding and stability. Structural modeling and visualization were performed using Swiss-PdbViewer (DeepView) 4.1.

DISCUSSION

In this report, the fetus presented with the main symptoms of hydrops fetalis, which had recurred from two previous pregnancies. Additionally, this fetus harbored a homozygous likely pathogenic variant of *SUMF1*: c.538T>A (p.Trp180Arg), having

inherited one mutant allele from each parent in an autosomal recessive manner.

The p.Trp180Arg substitution in SUMF1 potentially introduces significant alterations to the protein's structure and function. This mutation replaces tryptophan, a large, hydrophobic amino acid featuring an indole ring, with arginine, a hydrophilic and positively charged residue. Such a dramatic change in physicochemical properties at position 180 could disrupt the protein's local and global conformations. The introduction of a charged residue in place of a hydrophobic one may alter the protein's folding patterns, potentially exposing previously buried regions or causing the protein to misfold. Furthermore, the positive charge of arginine could introduce new electrostatic interactions, both intramolecularly and with surrounding molecules, potentially affecting the protein's stability, binding affinity, or catalytic activity. If position 180 is situated within or near the enzyme's active site or a critical structural domain, this substitution could directly impair SUMF1's catalytic function or its ability to interact with substrate molecules. Consequently, this amino acid change has the potential to significantly compromise the enzyme's role in sulfatase activation. This alteration may lead to reduced or loss of enzymatic activity, resulting in the accumulation of sulfatase substrates.

This variant of *SUMF1*: c.538T>A (p.Trp180Arg) was classified as a Variant of Uncertain Significance by ACMG (PP3 + PM5 + PM2) (Richards *et al.*, 2015). It was predicted to be "Pathogenic Strong" according to In-Silico Predictions (including BayesDel addAF - Pathogenic Strong – score: 0.5815; BayesDel noAF - Pathogenic Strong – score: 0.5975; MetaRNN - Pathogenic Strong – score: 0.9897; REVEL - Pathogenic Strong – score: 0.973; MetaLR - Pathogenic Moderate – score: 0.9672; MetaSVM - Pathogenic Moderate – score: 1.0949) (Dong *et al.*, 2015; Liu *et al.*, 2011; Liu *et al.*, 2016). This variant was previously reported once in ClinVar and was classified as a Variant of "Uncertain Significance". The variant is absent from population databases (gnomAD) and has not been previously reported in the literature in individuals with *SUMF1*-related conditions.

Based solely on the above classifications provided, it is difficult to conclusively determine the pathogenic nature of the detected variant of *SUMF1:* c.538T>A (p.Trp180Arg) in the fetus. However, we noted the mother's significant obstetric history with two prior pregnancies affected by hydrops fetalis. There is no molecular evidence of SNVs or indels in the two previous fetuses affected by hydrops fetalis, as genetic examinations were limited to chromosomal analysis in terms of number and structure (karyotyping) without sequence-level genetic testing. The current pregnancy presented with hydrops fetalis, similar to the two previous pregnancies in this family. In the current case, we identified a rare homozygous *SUMF1* variant (c.538T>A, p.Trp180Arg). While molecular evidence from the previous pregnancies is unavailable due to limitations in genetic testing performed, the recurrence of the same phenotype (hydrops fetalis) across three pregnancies in the same family is consistent with an autosomal recessive inheritance pattern. The variant's classification remains uncertain based on currently available population data; however, the phenotypic recurrence pattern observed in this family, together with the carrier status of both parents, aligns with the expected

inheritance pattern for a pathogenic variant in *SUMF1*. Additional functional studies would be beneficial to definitively establish the variant's pathogenicity.

In this case, we also suspected consanguinity or residence within the same endemic region for a circulating pathogenic variant between the parents, despite an unclear history of consanguinity based on their accounts. The recurrence of hydrops fetalis across multiple pregnancies raised suspicion for an autosomal recessive condition, which is more likely to manifest in offspring of consanguineous unions or populations with a higher prevalence of specific pathogenic founder variants. Therefore, performing prenatal diagnosis and identifying the causative genetic variant was critical for accurate recurrence risk counseling and reproductive planning for this couple.

We performed prenatal diagnosis for this fetus using CVS. CVS is a technique that enables early diagnosis of fetal abnormalities in the first trimester, providing results up to 4-5 weeks earlier than amniocentesis. CVS shortens the waiting period for prospective parents and avoids potential complications associated with the later termination of an affected pregnancy. At the Fetal Medicine Center of Hanoi Obstetrics and Gynecology Hospital, we have implemented prenatal diagnosis via CVS for pregnancies at high risk of chromosomal abnormalities or monogenic disorders. Specifically, in this report, we utilized CVS to diagnose a case of hydrops fetalis detected at 12 weeks gestation, in which the mother had a history of two prior pregnancies affected by hydrops fetalis. Early-onset hydrops fetalis portends a poor prognosis, with potential for intrauterine fetal demise or stillbirth prior to the typical gestational age for amniocentesis at 16 weeks. Therefore, early prenatal diagnosis was paramount in this case, and CVS was the appropriate diagnostic approach. The results obtained were unequivocal, and performing gene sequencing on the cultured chorionic villus sample was entirely reasonable, without any technical difficulties in execution or interpretation.

Limitations of the study

A limitation of this study is the absence of sulfatase enzymatic activity testing to confirm the impact of the homozygous *SUMF1* variant (c.538T>A, p.Trp180Arg) on sulfatase function. Due to time constraints and technical challenges in assessing enzymatic activity at the early gestational stage, such testing was not feasible before the pregnancy termination decision. This limits our ability to definitively link the variant to NIHF or rule out coincidental findings. However, Fraldi *et al.* (2007) and Cosma *et al.* (2003) established *SUMF1* as a critical regulator of all sulfatase activities, with pathogenic mutations causing global sulfatase deficiency. While enzymatic data would be ideal, the genetic findings and SUMF1's established role strongly suggest multiple sulfatase deficiency as the likely cause of NIHF in this case.

CONCLUSION

In this case report, we detected a homozygous missense variant in the *SUMF1* gene in a fetus presenting with NIHF through early prenatal diagnosis using CVS. This result underscores the importance of advanced genetic testing in cases of complex fetal conditions, especially when there is a history of recurrent hydrops fetalis. The findings

support the notion that early invasive diagnostic techniques can provide critical insights for informed decision-making and management of pregnancies at risk for genetic disorders.

This case emphasizes the need for continued research into the genetic underpinnings of multiple sulfatase deficiency and related conditions, which remain poorly understood.

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CONFLICT OF INTEREST

All financial, commercial, or other relationships that might be a potential conflict of interest must be disclosed. If no such relationship exists, authors will be asked to confirm the following statement: "The authors declare that there is no conflict of interest.".

ETHICAL APPROVAL STATEMENT

This study was approved by the Medical Ethics Council of Hanoi Obstetrics and Gynecology Hospital (Approval number: 2157/QD-PSHN, 14/12/2022). Exome sequencing was performed as a clinical service under appropriate clinical consent. Retrospective data collection from medical records was granted with a waiver of informed consent.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from the parents of the fetuses for genetic testing and the use of data in this study. All clinical information presented in this report has been de-identified to protect patient privacy.

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