Case Report

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Blended Phenotypes From a *SERPINA 11* Pathogenic Variant Over Underlying Immune Fetal Hydrops: A Rare Case Report and Literature Review

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Abstract

Fetal hydrops can stem from immune or nonimmune causes. Immune causes often involve red cell alloimmunization, whereas nonimmune causes encompass structural malformations, aneuploidy, infections, lymphatic system disorders, genetic syndromes, and more. In a rare and complex case, we encountered a fetal hydrops presentation characterized by blended phenotypes, indicating both a genetic and an underlying immune etiology. The mother, Rhesus negative, presented with a history of adverse obstetric events. At 21 weeks, the current fetus was diagnosed with hydrops. Maternal blood tests unveiled Rhesus alloimmunization, featuring a positive indirect Coombs test at a 1:512 dilution and the presence of anti-D, anti-C, and anti-E antibodies. Fetal blood sampling revealed an O-positive blood group with a hemoglobin level of 10 gm/dL. Despite administering intrauterine transfusion to the fetus, there was no improvement; instead, the fetal hydrops worsened, accompanied by the emergence of nuchal and axillary masses. Exome sequencing of fetal DNA revealed the fetus was homozygous for a pathogenic variant in the *SERPINA11* gene and compound heterozygous for a pathogenic variant in the PIEZO1 gene. Furthermore, the combination of pathogenic variants in *SERPINA11* and *PIEZO1* genes has not been described in cases of fetal hydrops before. This case posed significant challenges in management due to the concurrent presence of both immune and nonimmune hydrops. We describe some of the diagnostic challenges faced in clinical management of this case.

Keywords: Fetal chylothorax; *SERPINA11* gene; Rhesus alloimmunization; Fetal hydrops; Prenatal diagnosis; Blended phenotypes

Introduction

The etiology of fetal hydrops can be classified as either immune or nonimmune. Immune-related causes include red cell alloimmunization, most notably Rhesus alloimmunization, which can lead to severe fetal anemia and subsequently fetal hydrops. Common nonimmune etiologies include fetal structural malformations, aneuploidy, infections, and lymphangiectasis, many of which are associated with underlying genetic aberrations. Genetic syndromes account for approximately 15% of nonimmune hydrops fetalis.[1] Here, we present a rare case of fetal hydrops with a blended phenotype resulting from a genetic etiology and an underlying immune pathology, specifically Rhesus alloimmunization. Informed written consent was obtained from the couple. This case also highlights the challenges in managing the coexistence of immune and nonimmune hydrops.

Case presentation

A 33-year-old woman, in a third-degree consanguineous marriage, presented with a history of poor obstetric outcomes. She was gravida 5, para 2, with no living children. Her obstetric history included two first-trimester miscarriages, one neonatal death, and one intrauterine fetal demise. A morphology scan in her current pregnancy revealed fetal hydrops. Until now, her pregnancy had been uneventful with no reported symptoms. Her vital signs were stable, and her uterine size was consistent with a 22-week gestation. Her blood type was O negative, whereas her spouse was Rhesus positive (O positive).

In her previous obstetric history, her first two pregnancies ended in first-trimester miscarriages. During her third pregnancy, she delivered a male infant at term, weighing 2.4 kg. The infant had genital and bilateral lower limb swelling, later diagnosed as congenital lower limb lymphedema (Fig. 1). Unfortunately, the child died of sepsis at 1.5 years of age. After delivery, the mother developed a rectovaginal fistula, which required surgical repair.

During her fourth pregnancy, the fetus was diagnosed with exomphalos and subsequently died at 33 weeks of gestation, leading to the delivery of a macerated stillborn via lower-segment cesarean section. Notably, the mother had not received anti-D prophylaxis in any of her previous pregnancies. In the current pregnancy, the nuchal translucency scan was normal; however, combined first-trimester screening for aneuploidy was not performed. A morphology scan at 21 weeks revealed

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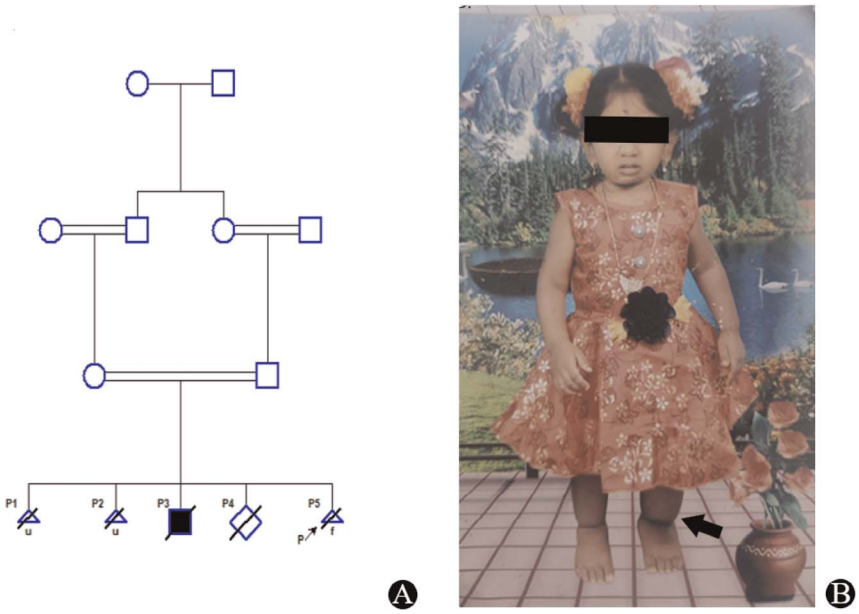

Figure 1. A three-generation pedigree with consanguinity. A Three-generation pedigree chart depicting consanguinity within the family. B Picture of previous child affected with congenital lymphedema at 18 months of age.

fetal hydrops, prompting a referral to our center. A detailed ultrasound assessment at our facility confirmed a single live intrauterine pregnancy with fetal biometry consistent with the gestational age. The fetus presented with right-sided pleural effusion, minimal ascites, and 7 mm of subcutaneous edema (Figs. 2A, B). There were no other structural anomalies, and the fetal middle cerebral artery peak systolic velocity (MCA PSV) was within normal limits at 18 cm/s (<1.5 multiples of the median (MoM)).

Maternal blood investigations confirmed her blood group as O-negative, with a positive indirect Coombs test at a 1:512 dilution. Additionally, maternal red cell antibodies, including anti-D, anti-C, and anti-E antibodies from the Rhesus system, were detected. These findings led to a diagnosis of immune hydrops secondary to Rhesus isoimmunization.

A cord blood sample from the fetus revealed an O-positive blood group, a positive direct Coombs test (4+), and a hemoglobin level of 10 g/dL, indicative of anemia. The fetal karyotype was normal, and fetal DNA was sent for exome sequencing. An intrauterine transfusion (IUT) with 50 mL of fresh O-negative red cells was performed, raising the posttransfusion hemoglobin to 17 g/dL. This intervention was repeated after 48 hours. However, subsequent scans showed worsening subcutaneous edema, bilateral hydrothorax, and the development of axillary and nuchal masses (Figs. 2C, D), whereas the fetal MCA PSV remained normal. The combination of these findings—worsening fetal hydrops despite IUT and persistently normal fetal MCA PSV (<1.5 MoM)—prompted us to reconsider the initial diagnosis.

Given the lack of improvement in fetal hydrops following IUT, alternative diagnoses were considered. Fetal thoracocentesis revealed characteristics consistent with chylothorax, including a total leukocyte count of 350/μL with a predominant lymphocyte count of 94%. To address the hydrothorax, a thoracoamniotic shunt was placed, but the procedure was complicated by shunt migration.

Exome sequencing of fetal DNA provided further insight, identifying homozygosity for a pathogenic variant in the *SERPINA11* gene and compound heterozygosity for a *PIEZO1* variant (Table 1). The couple was given comprehensive counseling regarding the poor prognosis due to the presence of both immune and nonimmune hydrops, conditions that were progressively worsening. Sanger sequencing confirmed that the parents were heterozygous carriers of the *SERPINA11* variant as well as the *PIEZO1* variant (Table 1).

At 26^{+5} weeks, the patient presented with intrauterine fetal demise and subsequently delivered a macerated stillborn male infant weighing 1.5 kg. An autopsy revealed gross features consistent with hydrops and extramedullary hematopoiesis (Figs. 3A, B).

The couple received counseling, highlighting the increased risk of recurrence due to a combination of immune and nonimmune etiologies. They were informed about various reproductive options, including in vitro fertilization with Rhesus-compatible donor gametes, the use of a gestational surrogate to minimize the risk of maternal alloimmunization, preimplantation genetic diagnosis, and adoption as alternative family planning strategies.

Discussion

We present a case of a 33-year-old woman with a history of recurrent pregnancy losses and adverse perinatal outcomes, complicated by Rhesus isoimmunization. The patient is in a third-degree consanguineous marriage, and her family pedigree demonstrates a significant degree of consanguinity (Fig. 1). Rapid exome sequencing of the fetal blood sample identified variants in two distinct disease-causing genes associated with nonimmune hydrops fetalis (Table 1):

1. *SERPINA11* gene (exon 2, c.436C > T, p.Arg146Ter, homozygous nonsense), which has recently been recognized as a candidate gene for nonimmune hydrops fetalis[2]

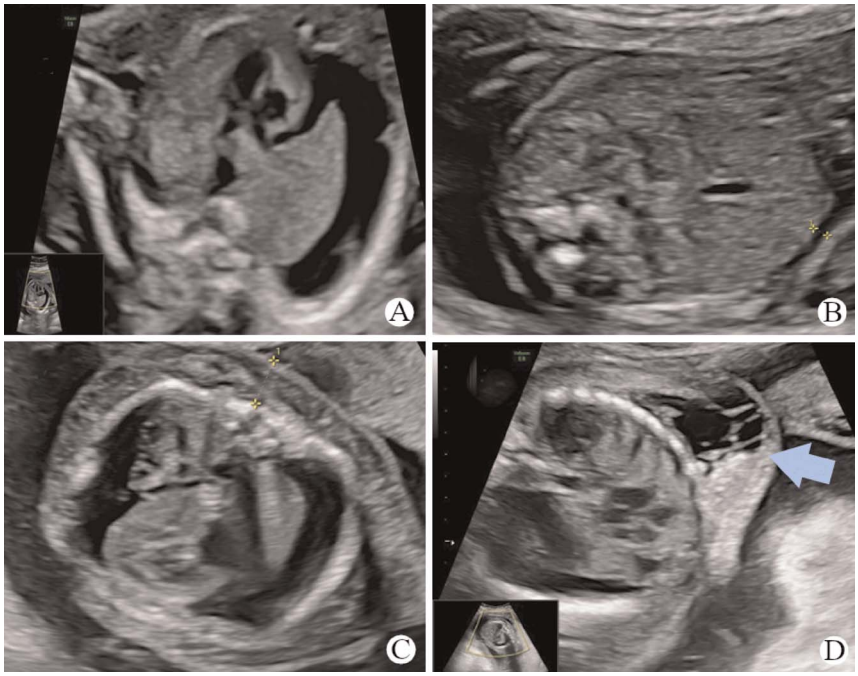

Figure 2. Two-dimensional ultrasound images of the fetus before and after intrauterine transfusion. A&B The upper panel shows ultrasound images of the fetal thorax (A) with right-sided hydrothorax and the fetal abdomen (B) with minimal fetal ascites before IUT. C&D The lower panel images from subsequent scans (after IUT) depict bilateral hydrothorax (C) and the appearance of axillary lymphatic masses (D), indicated by the arrow. IUT: Intrauterine transfusion.

2. *PIEZO1* gene (exon 16, c.436C > T, p.Met711Lys, exon 40, c.5691_5705del, p.Glu1898_Glu1902del, compound heterozygous), associated with lymphatic malformation 6 (OMIM #616843)

Segregation analysis confirmed that both parents were heterozygous carriers of the aforementioned variants in transconfiguration. The *SERPINA11* nonsense variant results in a premature truncation of the protein at codon 146 in the 422-amino-acid chain. This novel variant is classified as pathogenic according to current American College of Medical Genetics guidelines.[3]

The *PIEZO1* exon 16 and exon 40 variants have minor allele frequencies of 0.02% and 0.0056%, respectively, in

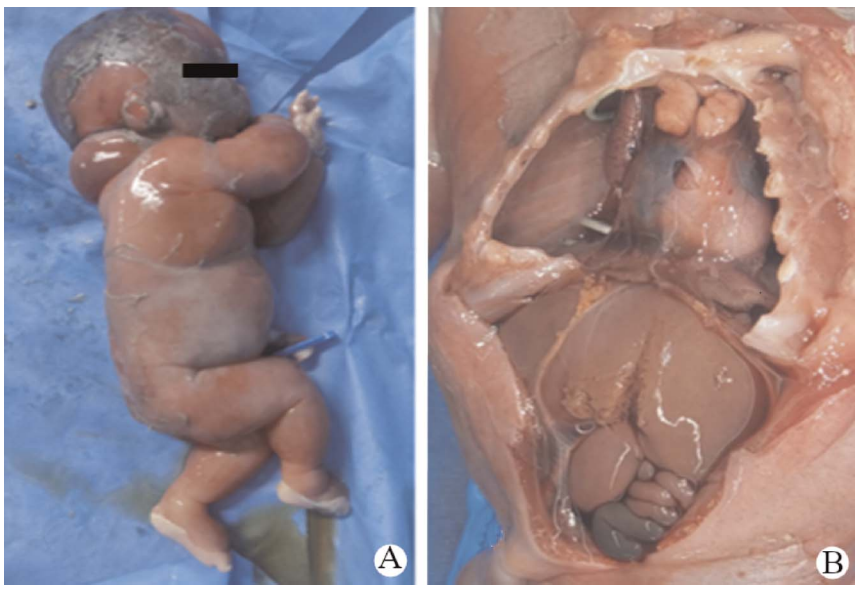

Figure 3. A Autopsy image of the stillborn fetus with axillary and nuchal masses. B The dissected abdominal and thoracic cavities of the stillborn fetus.

Table 1

Rapid exome sequencing of the fetus.

Gene (transcript)	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classification
SERPINA11 (ENST00000334708.4)	Exon 2	c.436C > T (p.Arg146 > Ter)	Homozygous	No OMIM entry	—	Pathogenic
PIEZO1 (ENST00000301015.14)	Exon 16	c.2132 T > A (p.Met711Lys)	Compound heterozygous	Lymphatic malformation 6 (OMIM#16843)	Autosomal recessive	Uncertain significance
	Exon 40	c.5691_6705del (p.glu1898_Glu1902del)	Compound heterozygous	Lymphatic malformation 6 (OMIM#16843)	Autosomal recessive	Uncertain significance

OMIM: Online Mendelian Inheritance in Man; —: Not applicable.

the 1000 Genomes database. Both variants are classified as variants of unknown significance due to either insufficient or conflicting evidence. However, the couple's second male child was affected by bilateral lower limb lymphedema in early infancy, a phenotype consistent with *PIEZO1* lymphatic malformation type 6 (OMIM#616843).

Our case draws parallels with previously reported *PIEZO1* variants, where pregnancies complicated by hydrops fetalis often resulted in intrauterine demise or severe postnatal lymphedema in surviving neonates.[4]

Fetal autopsy findings corroborated the ultrasound results, revealing asymmetrical subcutaneous swelling and edema in the nuchal regions and extremities. There was also visceral involvement, including bilateral pleural effusion and ascites. Histopathological examination confirmed extramedullary hematopoiesis consistent with fetal anemia, aligning with findings in autopsies of previously reported *PIEZO1*-related hydrops fetalis cases.[5]

In a concurrent Indian study, an autopsy report described milder visceral involvement, along with pleural and pericardial blebs, in a *SERPINA11* phenotype associated with a homozygous missense variant.[6] However, there are no prior case reports documenting an overlap between *PIEZO1* and *SERPINA11* phenotypes, making it challenging to determine the exact consequences of a blended phenotype involving variants in both genes.

The function of the *PIEZO1* protein, a calcium-permeable ion channel, in the development and maintenance of lymphatic valves has been well established.[7] Homozygous or compound heterozygous loss-of-function variants in *PIEZO1* are known to cause lymphatic malformation 6 (OMIM#616843), a congenital lymphatic dysplasia characterized by widespread multisegmental lymphedema and systemic involvement, often leading to in utero deaths. Further research is needed to provide a clearer understanding of the *SERPINA11* protein's function and its potential impact when coexisting with *PIEZO1* variants.

The adverse fetal outcomes appear to be due to a combination of genetic etiology and Rhesus alloimmunization. The recurrence risk for either of the two autosomal recessive conditions, or both, is compounded to 43.75%.[8]

Blended phenotypes of genetic disorders have been previously documented in medical literature,[9] but this case is notable for presenting a rare instance where a blended phenotype is exacerbated by an underlying nongenetic immune cause, namely, Rhesus isoimmunization.

Clinical features supporting the diagnosis of immune hydrops include the mother's negative blood group and the fetus's positive blood group; lack of anti-D prophylaxis in any of the mother's previous pregnancies; maternal blood tests showing anti–red cell antibodies of the Rhesus system, along with a positive indirect Coombs test; and cord blood analysis confirming fetal anemia (Hb 10 g/dL) with a strongly positive direct Coombs test.

Clinical characteristics favoring a genetic etiology include a history of third-degree consanguinity; a previous child affected by lower limb lymphedema; in the current pregnancy, MCA PSV not indicating fetal anemia; worsening of fetal hydrops despite IUT; and the presence of pathogenic variants in the *PIEZO1* and *SERPINA 11* genes, known to be associated with fetal hydrops, confirmed by parental validation.

Conclusion

In cases where hydrops worsens after IUT in suspected immune hydrops, it is essential to consider nonimmune causes, such as genetic syndromes. Prenatal diagnosis and genetic counseling are vital for the effective management of such complex pregnancies.

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None.

Conflicts of Interest

None.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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