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CLINICAL REPORT

A novel heterozygous deletion in *ABCB4* gene in a Chinese family with intrahepatic cholestasis of pregnancy, neonatal hyperbilirubinemia, and cholelithiasis: Case reports and literature review

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Abstract

Background: *ABCB4* gene (OMIM *171060) variant is associated with a wide clinical spectrum of hepatobiliary diseases, including familial intrahepatic cholestasis of pregnancy (ICP), progressive familial intrahepatic cholestasis type 3 (PFIC3), and neonatal hyperbilirubinemia due to impaired protection of the bile duct. The majority of reported cases, however, were missense or nonsense variants, with few deletion variant findings in the Chinese population.

Method: We performed whole genome sequencing and confirmed it with Sanger sequencing of the proband infant and his families. Clinical courses and laboratory results were documented and collected from the proband infant and his mother. We also reviewed other published cases related to genetic variants in *ABCB4* in the Chinese population.

Results: A 26-year-old Chinese female (II.2) who had recurrent intrahepatic cholestasis of pregnancy and her 49-day-old son (III.4) who had hyperbilirubinemia, both presented with extremely elevated total bile acid, cholestatic dominant pattern liver function abnormalities. They were able to stay relatively stable with mild pruritus on ursodeoxycholic acid treatment. After ruling out other possibilities, genetic sequencing revealed a diagnosis of heterozygous deletion variant NM_018849.3:c.1452_1454del (NP_061337.1:p.Thr485del) in *ABCB4*, which was not reported before, in the symptomatic mother (II.2), index patient (III.4), and the symptomatic grandmother (I.2). This variant resulted in clinical spectrums of ICP, neonatal hyperbilirubinemia, and cholelithiasis in our pedigree.

Conclusion: We reported a novel heterozygous deletion variant of the *ABCB4* gene in a Chinese family, as well as a literature review of *ABCB4*-related disorders. We aim to facilitate healthcare professionals to better understand genetic factors as an uncommon cause of hepatobiliary diseases, as well as improve therapeutic strategies in challenging clinical situations such as pregnancy and neonatal care.

KEYWORDS

ABCB4, case report, heterozygous deletion variant, intrahepatic cholestasis of pregnancy (ICP), neonatal hyperbilirubinemia

1 | INTRODUCTION

The adenosine triphosphate-binding cassette 4 (*ABCB4*) gene (OMIM *171060), also known as the multidrug resistance 3 (*MDR3*) gene, is located on human chromosome 7q21.12 and encodes the hepatic membrane-associated transport protein ABCB4/MDR3, which acts by translocating phospholipids from the inner to the outer leaflet of the canalicular membrane (Sticova & Jirsa, 2020). The variant of *ABCB4* may result in a wide clinical spectrum of diseases such as familial intrahepatic cholestasis of pregnancy (ICP), progressive familial intrahepatic cholestasis type 3 (PFIC3), low phospholipid-associated cholelithiasis (LPAC) syndrome, and neonatal cholestasis due to the weakened function of avoiding cholesterol gallstone formation, and impaired phospholipid neutralization of the detergent effect of hydrophobic bile salts (Pasmant et al., 2012; Stättermayer et al., 2020).

Previous studies have identified a wide range of *ABCB4* variants in cholestasis diseases. The majority of reported cases, however, are missense or nonsense variants, with only a few deletion variant findings in the Chinese population (Bai et al., 2021; Dixon et al., 2017; Wu et al., 2020). Hereby, we describe two cases: a 26-year-old Chinese female with recurrent ICP, and her newborn child with neonatal hyperbilirubinemia, both of whom were determined to be heterozygous for a deletion variant of the *ABCB4* gene (the pedigree is presented in Figure 1). The case reports aim to promote awareness of *ABCB4*-related familial cholestasis diseases and to improve therapeutic strategies in challenging clinical situations such as pregnancy and neonatal care.

2 | METHOD

2.1 | Ethical compliance

This study was approved by the Ethics Review Board of the First Affiliated Hospital, School of Medicine, Zhejiang University (Reference No. 2022-231) and with the Helsinki Declaration (as revised in 2013). Written informed consent for participation and publication was obtained from the patient or the patient's guardians.

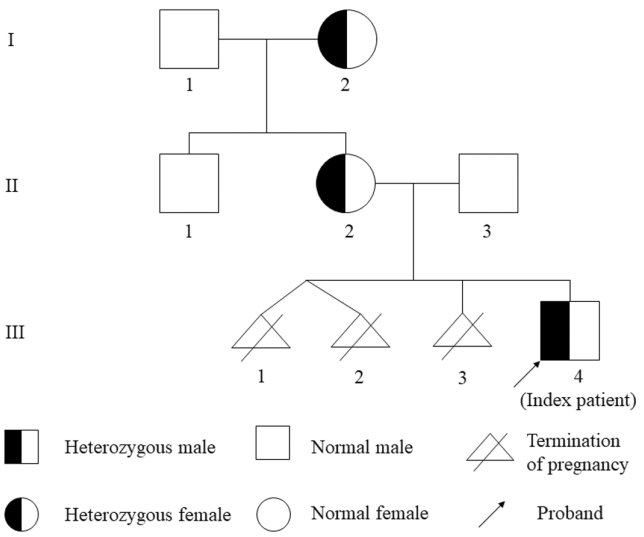

FIGURE 1 Pedigree chart of the patient's family.

2.2 | Genetic sequencing

Genomic DNA was extracted from blood samples of the proband and their families (*ABCB4*: GenBank accession number: NG_007118.3 for genomic DNA, NM_000443.4 for mRNA isoform A, NM_018849.3 for mRNA isoform B, NM_018850.3 for mRNA isoform C). Whole genome sequencing (Illumina Nextseq 500) was initially used to identify potential SNP\INDEL mutations in comparison with hg19. Following Sanger sequencing was used as a confirmatory tool for the pathogenic or likely pathogenic genetic mutation.

2.3 | Literature review

We comprehensively reviewed *ABCB4*-related literature published earlier than June 2023 on the databases of PubMed and Google Scholar using the keywords "ABCB4 OR ABCB4 gene OR ABCB4 variant* OR MDR3", "case report OR case series OR case*". Studies diagnosed with genetic sequencing with clinical diagnosis, full clinical presentation, and identified specific *ABCB4* variants were included. Editorials, conference abstracts, systematic reviews or meta-analysis, cases without ascertained clinical diagnosis or not confirmed by genetic testing were excluded.

3 | RESULTS

3.1 | Case reports

3.1.1 | The mother with severe ICP (II.2)

A 26-year-old female, gravida (number of pregnancy) 3, para (number of giving birth to a viable child) 0, in the 7th week of her third pregnancy, presented with generalized skin pruritus, fatigue, and anorexia. No jaundice, nausea, vomiting, diarrhea, abdominal pain, or fever were noted. She had two similar previous episodes in the past. The first episode presented 4 years earlier in the context of pruritus, moderate jaundice, fatigue, abnormal liver function test, and ultrasound-confirmed intrahepatic bile duct stones with bile duct dilation in the first trimester of gravida 1. And the second episode occurred 3 years earlier during the first trimester of gravida 2. Both had been treated with the termination of pregnancy and conservative drug therapy, and the patient was stable during the non-pregnant period. Family history showed that her mother (I.2) underwent cholecystectomy for gallstones in the past, but a thorough history could not be obtained.

Several days prior to her presentation, she experienced vaginal bleeding accompanied by mild pruritus, a liver function test was abnormal, and she was initially diagnosed with threatened abortion at the local hospital, where tocolysis therapy had begun. On the day of the presentation, the patient was extremely anxious about her pregnancy and expressed a strong desire for a healthy baby, and she was admitted to our hospital. Her physical examination was normal, and the fetus's condition was reassuring. The initial laboratory studies showed a cholestatic dominating pattern of liver function abnormality (Appendix S1). On further testing, anti-nuclear antibody, anti-mitochondrial antibody, anti-liver-kidney-microsomal antibody, anti-smooth muscle antibody, and ceruloplasmin were all negative or within normal limits. A viral serology screen was negative for hepatitis A, B, C, D, E, and HIV. Abdominal ultrasound (Figure 2) revealed intrahepatic bile duct stones with slight bile duct dilation, and gallbladder stones, but no evidence of portal hypertension or other vascular abnormalities. Original reports with no obvious abnormalities were not presented here. Further evaluation by computed tomography (CT) or magnetic resonance cholangiopancreatography (MRCP) was not suggested considering the potential hazard to the fetus. A diagnosis of ICP was obtained based on several pruritus, significantly elevated total bile acids and cholylglycine, and spontaneous relief following a pregnancy termination. The patient was therefore started on S-adenosyl methionine 500 mg twice daily, polyene phosphatidylcholine 456 mg three times daily, glycyrrhizinic acid 80 mg once daily, and reduced glutathione 1200 mg once daily. The patient gradually improved clinically and biochemically over the following days and was discharged on day 14 of hospitalization. Thereafter, the patient was regularly followed up at the clinic with continuing pharmacological therapy of S-adenosyl methionine and ursodeoxycholic acid 250 mg three times daily, which was prescribed since the beginning of the second trimester in accordance with the medication instructions, and tocolysis therapy. Her condition remained generally stable after discharge, with only mild pruritus, and her biochemical parameters were kept at a low level for the majority of the time, with the exception of one rebound induced by medication self-withdrawal.

In the 32nd week of pregnancy, the patient presented with exaggerated and unremitting pruritus, involuntary tremors of upper limbs, abdominal distension, and fetal tachyarrhythmia. Worsening liver function was also noted. Uterine contractions could not be stopped despite extensive tocolytic treatment, and birth was unavoidable. To ensure the safety of the fetus, an emergency cesarean section was performed following antenatal dexamethasone administration. The surgery course was uneventful, and the newborn was transferred to the neonatal intensive care unit (NICU) following the procedure. S-adenosyl methionine and ursodeoxycholic acid were discontinued after the operation; liver function improved on postpartum day 5 and remained fully within the normal range in the next four weeks.

3.1.2 | The neonate with neonatal hyperbilirubinemia (index patient, III.4)

A 47-day-old male neonate was admitted to the pediatric department for diarrhea and jaundice. He was delivered 32 weeks prematurely by cesarean section with a birthweight of 2.20 kg, and an Apgar score of 7/9/10, to a mother with severe ICP. Following delivery, the neonate required supportive care with a mechanical ventilator, CPAP, blue light therapy, UDCA drug therapy, and nutrition support for 20 days in the NICU for neonatal respiratory distress, neonatal hyperbilirubinemia, cholestasis, and was subsequently discharged with stable vital signs, improved appetite, nutrition status, and clinically improved laboratory parameters (total bilirubin 87 μmol/L, direct bilirubin 41 μmol/L). Ursodeoxycholic acid 25 mg twice daily was prescribed at home. However, on day 36 of life, severe diarrhea with yellow-green loose stool with odor 4–5 times per day, fat granules were investigated at the local hospital, and no mucus or blood was noted. He

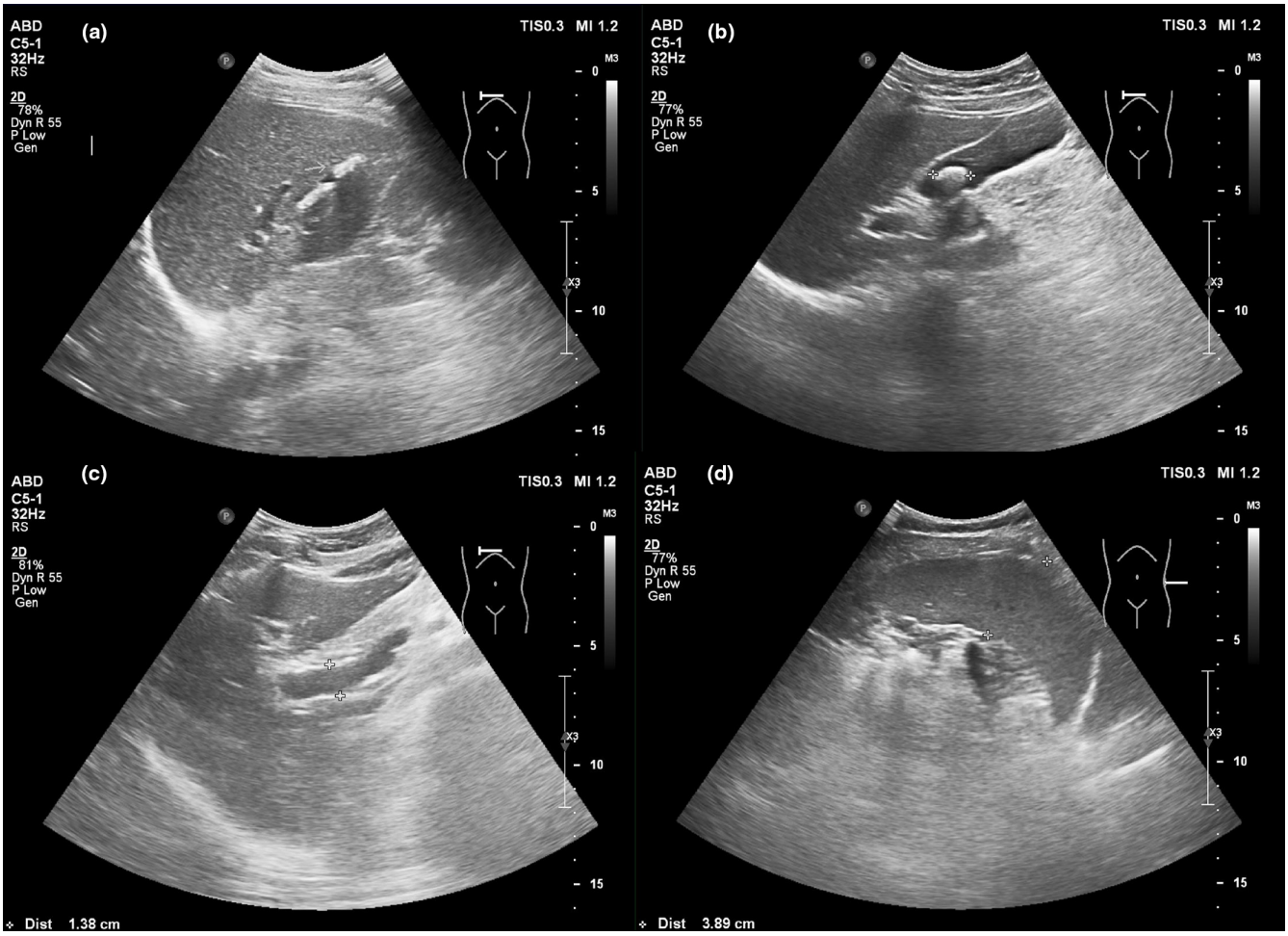

FIGURE 2 Abdominal ultrasound results of the symptomatic mother (II.2). (a) Intrahepatic bile duct stones (white arrow) with slight bile duct dilation; (b) Stone in the gallbladder (diameter 1.57 cm); (c) Portal vein (width 1.38 cm); (d) Splenic hilum thickness (3.89 cm).

was then referred to our hospital on day 47 of life once again.

At presentation, he was afebrile, alert, and had a high-pitched cry. He experienced no vomiting, rash, or cough. Physical examination showed a normal temperature of 37.3°C, a heart rate of 148 bpm, a respiratory rate of 40 bpm, a blood pressure of 91/67 mmHg, and mild skin jaundice. There was no abdominal tenderness. Liver function test revealed elevated total bilirubin, alkaline phosphatase, gamma-glutamyl transpeptidase, and total bile acid (Appendix S1). Ultrasound revealed no structural biliary duct abnormalities. In suspicion of genetic causes, the boy and his family members underwent genomic sequencing after obtaining written informed consent. Initial management included ursodeoxycholic acid 50 mg once daily, S-adenosyl methionine 150 mg once daily, diosmectite, probiotics, and appropriate fluid and electrolyte support. The patient was clinically and biochemically better after one week of hospitalization, and he was then discharged home on ursodeoxycholic acid therapy.

Two months later, whole genomic sequencing (Illumina Nextseq 500) followed by Sanger sequencing as a confirmatory tool identified a heterozygous NM_018849.3:c.1452_1454del variant in exon 13 of *ABCB4* gene in the boy (III.4), the boy's mother (II.2), and the boy's maternal grandmother, who reported a history of cholelithiasis and cholecystectomy. A genetic disorder of the *ABCB4* gene deletion variant characterized by intrahepatic cholestasis of pregnancy, neonatal hyperbilirubinemia, and cholelithiasis was confirmed in this Chinese family. The mother (II.2) maintained regular outpatient visits with our team for the subsequent three years. During this period, her liver function test revealed no significant abnormalities, and her liver ultrasound exhibited findings consistent with prior examinations, showing minimal dilation of intrahepatic bile ducts and the presence of gallstones. Although she verbally informed us that her son (III.4) was doing fine, no supporting blood test or imaging data were provided. Subsequently, this family was lost to follow-up due to their relocation to another province.

3.2 | Literature review

More than 70 cases of *ABCB4* variants were reported worldwide, accounting for a wide spectrum ranging from PFIC3 to mild conditions like intrahepatic cholestasis of pregnancy, gallstone diseases, and elevated liver function tests. It was first described by Deleuze et al. that the absence of *ABCB4/MDR3* mRNA was detected in two patients with progressive familial intrahepatic cholestasis in 1996 (Deleuze et al., 1996). Later, based on the modern genetic techniques, numbers of homozygous or compound-heterozygous variants were confirmed to result in PFIC3 by either premature truncation of the ABCB4/MDR3 protein or a total failure of protein production (Stättermayer et al., 2020). Milder phenotypes were also frequently reported to be associated with *ABCB4* variants. For instance, two missense variants specific for drug-induced cholestasis or hepatocellular injury were discovered in a cohort study by Lang et.al (Lang et al., 2007). In a family with multiple members with *ABCB4* variant and chronic liver disease, all female members suffered from ICP, including both homozygotes and heterozygotes (Gotthardt et al., 2008). Genome-wide association study in a large-scale Icelandic population analyzed the correlation between common *ABCB4* variants and the presence of liver diseases, the result indicated that a missense variant and a frameshift insertion variant were related to gallstones, ICP, cirrhosis, liver cancer, gallbladder cancer (Gudbjartsson et al., 2015).

ABCB4 variant-related diseases, clinical presentations, and genetic variants both in the Chinese population and non-Chinese population were reviewed and summarized in Table 1. Biliary atresia, PFIC3, intrahepatic stones, cholestasis, and intrahepatic cholestasis of pregnancy have been identified as common *ABCB4* variant-related diseases. Among the reported variants, missense made up the majority; additional variants included nonsense, splicing, frameshift, synonymous, etc.

4 | DISCUSSION

This case report described a novel *ABCB4* heterozygous deletion variant in a 26-year-old female presenting with cholelithiasis, severe intrahepatic cholestasis, and a 47-day-old neonate presenting with hyperbilirubinemia. The clinical presentation with generalized skin pruritus aggravated during pregnancy and alleviated by terminated pregnancy, significantly elevated total bile acid, ALP, GGT, ultrasound-confirmed intrahepatic bile duct dilation, responsiveness to ursodeoxycholic acid therapy, and positive family history prompted us to consider the possibility of a genetic disorder affecting the hepatobiliary system. The differential diagnosis of cholestasis and abnormal liver function tests in adults and children is broad. Common causes were ruled out, including viral or bacterial infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, endocrine problems, and vascular anomalies. There is no evidence of drug-induced liver injury, malnutrition, or neoplasm. We also ruled out other uncommon causes such as Wilson disease, hemochromatosis, and congenital anomaly of the biliary tract. To assess the likelihood of a genetic attribution, we performed genomic sequencing analysis and revealed a deletion heterozygous variant in exon 13 of *ABCB4*, which was first reported to the best of our knowledge. Although high levels of total bile acid may be harmful to the fetus (Vasavan et al., 2021), the young mother showed a great wish for a healthy baby. Clinical data suggests that the advantages of UDCA exceed the dangers for the fetus (Chappell et al., 2012). After obtaining informed permission, we prescribed S-adenosyl methionine in the first trimester and UDCA in the second and third trimesters of pregnancy in line with the pharmaceutical recommendations for cholestasis, and tocolytic medications for extending the pregnancy.

The ABCB4/MDR3 protein acts as a lipid translocator that moves phosphatidylcholine from the inner to outer leaflet of the canalicular membrane for secretion into the bile (Sundaram & Sokol, 2007). It consists in 2 transmembrane domains (TMD), assumed to be involved in substrate specificity and secretion, and 2 cytoplasmic nucleotide-binding domains (NBD), highly conserved sequences with 8 motifs (A-loop 401, Walker-A 427–434, Q-loop 473–477, X-loop 522–528, Signature 531–536, Walker-B 551–556, D-loop 559–562, H-loop 583–587) for ATP binding and hydrolyzing (Gautherot et al., 2014; Stättermayer et al., 2020). ABCB4/MDR3 deficiency would lead to decreased secretion of biliary phospholipid, which played key effects on the neutralization of detergent effect of hydrophobic bile salts and stabilization of micelles (Jacquemin et al., 2001). Thus, patients with ABCB4/MDR3 deficiency are susceptible to biliary epithelium and canalicular membrane injury, cholesterol crystallization, generally presented as elevated level of GGT and/or transaminase within typical cases (Stättermayer et al., 2020). There are 407, 504, and 1689 *ABCB4* gene variants linked with clinical spectrum documented in HGMD, ClinVar, and gnomAD, including missense, nonsense, splice site, insertion, and deletion variants (The Human Gene Mutation Database, n.d.; GnomAD, n.d.; ClinVar, n.d.).. Homozygous or compound heterozygous individuals would lead to PFIC3, a rare autosomal recessive disease that can be lethal in the absence of liver transplantation. Other cholestatic or cholelithiasis diseases with less severe presentation, such as ICP, LPAC syndrome, and neonatal

TABLE 1 Literature review of *ABCB4* variants reported.

ABCB4 variant-related disease	Clinical presentation	*ABCB4* variants reported	References
Biliary atresia	Severe jaundice, pruritus, and pale stool	Exon 13 IVS13+6G>A/G	Zhang et al. (2019)
Progressive familial intrahepatic cholestasis type 3 (PFIC3)	Cholestasis, jaundice, liver fibrosis or biliary cirrhosis	Splicing (c.80+1G>C, c.136-2A>G, c.1560+2T>A) Missense (c.667A>G, c.874A>T, c.955G>C, c.959C>T, c.1195G>C, c.1429C>A, c.1436C>T, c1756G>T, c.1804G>T, c.2137G>A, c2177C>T, c2471T>C, c.2525T>C, c.2950C>T, c.3139G>C, c.3152T>C, c.3220G>A) Frameshift (c.2489insA, c.3139_3141delGCAinsCC, c.3143delA) Nonsense (c.2123G>A Synonymous (c.175C>T, c.504C>T, c.711A>T)	Bai et al. (2021), Cheng et al. (2022), Liu, He, et al. (2022), Saleem et al. (2020), Wu et al. (2020), Zhang et al. (2020), Zhu et al. (2022)
Biliary stones	Imaging (Ultrasound or CT or MRCP) confirmed gallstones, intrahepatic stones	Synonymous (no. 69233, G>A) Missense (p.Gly622Glu) Frameshift (p.Leu445Glyfs*22)	Gudbjartsson et al. (2015), Pan et al. (2015)
Cholestasis	Chronic intrahepatic cholestasis, elevated GGT, drug-induced cholestasis/hepatocellular injury	Missense (c.1376A>G, c.1745G>A, exon 18: 2290A>C-I764L and exon 25: 3245T>A-L1082Q, c.2362C>T) Frameshift (c.2077_2078delC, c.3825_3826delA,) Splicing (c.344+2_+3insT) Nonsense (c.139C>T)	Fang et al. (2012), Gotthardt et al. (2008), Lang et al. (2007)
Intrahepatic cholestasis of pregnancy (ICP)	Pregnant women with skin pruritus and abnormal liver biochemistry indexes	Missense (p.Ile377Asn, p. Lys386Glu, p.Gly527Glu, p. Trp708Ter, p.Asp972Asn, p.Ser1153Asn)	Liu et al. (2021), Liu, Zheng, et al. (2022)

cholestasis, usually appear in heterozygotes (Falguières et al., 2014). Furthermore, different patterns of variants were associated with distinct clinical presentations and outcomes (Saleem et al., 2020). Patients with monoallelic missense variant, which accounted for more than 70% of cases, usually have less severe clinical phenotypes than nonsense or deleted variants that cause loss of function and mRNA instability (Jacquemin et al., 2001).

The genetic sequencing that revealed NM_018849.3:c.1452_1454del causes a NP_061337.1:p.Thr485del heterozygous variant in our patients. The Thr-485del variant is located in the first NBD sparing the 8 motifs, but the exact pathogenesis of this variant remains clinically unvalidated (Lawson et al., 2008). Previous research on the ABCB1 gene discovered that the Q-loop motif may be involved in NBD:NBD and NBD:TMD communication (Storm et al., 2007; Urbatsch et al., 2000). Because ABCB4 and ABCB1 have up to 76% identity and 86% similarity in the amino acid sequence, we hypothesized that Thr485del might interact with the adjacent residues in the Q-loop motif, interfering with NBD:NBD and NBD:TMD communication (Temesszentandrási-Ambrus et al., 2023). We further examined the variant's predictive biological function using PROVEAN, which yielded a score of −12.907, indicating a deleterious effect (prediction cutoff = −2.5), and SIFT Indel, which yielded a predicted damaging effect (PROVEAN(Protein Variation Effect Analyzer), n.d.; SIFT (Sorting Intolerant From Tolerant) Insertion/Deletions, n.d.).

5 | CONCLUSION

In summary, *ABCB4*-related cholestasis disorder is a rare condition that can affect pregnant women and newborns, resulting in a range of clinical symptoms ranging from moderate cholelithiasis to high ICP or neonatal hyperbilirubinemia. These two cases would help healthcare professionals to better understand genetic factors as an uncommon cause of hepatobiliary diseases, especially in the clinical setting with long-term symptoms and positive family history. Aggressive termination of pregnancy in severe ICP is not always feasible, and medication treatment combined with thorough monitoring may be useful

in some situations; however, more data are needed to establish this.

AUTHOR CONTRIBUTIONS

Jianqin He made the diagnosis and treatment decisions and conceptualized the Case Report. Yang Zheng, Qunfang Rao, and Yiru Han contributed to the literature search, data collection, and data interpretation. Yang Zheng contributed to the manuscript preparation. Jianqin He supervised manuscript preparation and had the final responsibility to submit for publication.

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No funding.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are not publicly available but are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of Ethics Review Board of the First Affiliated Hospital, School of Medicine, Zhejiang University (Reference No. 2022-231) and with the Helsinki Declaration (as revised in 2013).

PATIENT CONSENT

Written informed consent to participate was obtained from the patient or patient's parents.

CONSENT FOR PUBLICATION

Written informed consent was obtained from the patient or patient's parents for publication of this case report and accompanying images.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not appliable.

CLINICAL TRIAL REGISTRATION

Not appliable.

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SUPPORTING INFORMATION

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

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