

Recurrent early-onset severe obstetric cholestasis in a patient with two variants in the ABCB4 gene

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Abstract

A 36-year-old patient presented with severe early-onset obstetric cholestasis on a background of having pre-term induction of labor at 33 weeks during her only previous pregnancy. The patient had significantly abnormal liver biochemistry with a bilirubin of 78 $\mu\text{mol/L}$, ALP of 318 u/L, ALT of 280 $\mu\text{mol/L}$, and bile acid levels of 420 $\mu\text{mol/L}$. The patient received ursodeoxycholic acid 750 mg 3 times a day, rifampicin 500 mg twice a day, aspirin 150 mg once a day, and metformin 500 mg 3 times a day. However, despite this, the patient still suffered from intractable pruritus and her bile acid level was still above the 100 $\mu\text{mol/L}$ target that the obstetrics team was aiming for to avoid early delivery at 32 weeks. Due to the nature and severity of her cholestasis, the patient had a number of investigations done postnatally including genetic analysis, which confirmed that the patient was heterozygous for a pathogenic variant of the ATP-binding cassette subfamily B member 4 gene (c.959C>T [p.Ser320Phe]) and also a variant of unknown significance (c.1679C>T [p.Thr560Met]).

Keywords: ABCB4; cholestasis; intrahepatic cholestasis of pregnancy; MDR3; ursodeoxycholic acid.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a gestation-specific liver disorder that is characterized by the onset of pruritus, usually from the third trimester of pregnancy, associated with abnormal liver test results and/or increased total serum bile acids and spontaneous relief, typically within 48 h after delivery.^[1]

ICP is not typically associated with ongoing hepatic impairment after pregnancy and the biochemical abnormalities normally resolve within 2–8 weeks of delivery; however, in the majority of women, ICP recurs in subsequent pregnancies.

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The etiology of ICP is complex and not fully understood; however, genetic analysis has identified a variety of different genes which may be implicated in the development of ICP.

ATP-binding cassette (ABC) subfamily ABC B member 4 (ABCB4), also known as multidrug resistance protein 3 (MDR3), encoded by ABCB4, is involved in biliary phospholipid secretion, transporting phosphatidylcholine from the inner to the outer canalicular membrane, and thereby protecting the hepatobiliary system from deleterious detergent and lithogenic properties of the bile which is amplified by the absence of phosphatidylcholine.^[2]

In this case report, we highlight a patient with recurrent severe ICP with genetic analysis confirming that the patient is heterozygous for a pathogenic variant of the ABCB4 gene (c.959C>T [p.Ser320Phe]) and also a variant of unknown significance (c.1679C>T [p.Thr560Met]).

Case Report

We report a 36-year-old female who has had recurrent and severe early-onset obstetric cholestasis. The patient is of Albanian ethnicity and is a non-smoker who rarely drinks alcohol and has no other medical history.

In 2014, during her first pregnancy, she developed severe pruritus and jaundice with bile acid levels of >100 $\mu\text{mol/L}$ resulting in pre-term induction of labor at 33 weeks. Following delivery, her symptoms resolved with subsequent normalization of her liver function tests.

Following this, there were no further medical concerns apart from an isolated episode of pruritus in 2017. The patient visited her general practitioner who performed blood tests which did show abnormal liver biochemistry – (bilirubin 25 $\mu\text{mol/L}$, AST 53 u/L, ALT 128 u/L, and AP 137 u/L). These blood results were not repeated or followed up on; however, the patient reports spontaneous resolution of her pruritus.

In 2022, during her second pregnancy, the patient started developing an almost identical clinical presentation to her first pregnancy with severe pruritus and jaundice starting from 17 weeks into her pregnancy. Due to her severe ICP during her first pregnancy, the patient was under the care of an obstetric medical physician alongside the obstetrics team at a tertiary hospital.

Her liver biochemistry peaked with a bilirubin of 78 $\mu\text{mol/L}$, ALP of 318 u/L, ALT of 280 $\mu\text{mol/L}$, and bile acid levels of 420 $\mu\text{mol/L}$. The patient at this point had an abdominal ultrasound which was normal, a normal GGT, negative hepatitis A, B, and C serology, evidence of previous exposure to CMV, EBV, and HEV, and negative AMA, ANA, SMA, and LKM-1 antibodies.

The patient was started on ursodeoxycholic acid 750 mg 3 times a day, rifampicin 500 mg twice a day, aspirin 150 mg once a day, and metformin 500 mg 3 times a day. Despite this, the patient complained of intractable pruritus; however, her bile acid levels did reduce to 112 $\mu\text{mol/L}$ which was just above the 100 $\mu\text{mol/L}$ target that the obstetrics team was aiming for to avoid early delivery at 32 weeks.

As the bile acid levels remained above the 100 level at 32 weeks, a decision was made to proceed with early delivery of the obstetrics team. Two weeks postnatally, her bilirubin had reduced to 28 and her ALP had gone down to 220, however, given that they had not normalized, the patient was referred to the hepatology team. All of her medications apart from the ursodeoxycholic acid had been stopped at this point due to the improvement in her pruritus.

Due to the severity of her recurrent ICP, a genetic analysis was done to look for any genetic abnormalities related to cholestasis. Genetic analysis confirmed that the patient is heterozygous for a pathogenic variant of the ABCB4 gene (c.959C>T [p.Ser320Phe]) and also a variant of unknown significance (c.1679C>T [p.Thr560Met]).

The c.959C>T (p.Ser320Phe) mutation is rare in the general population and has been found in patients with progressive familial intrahepatic cholestasis type 3 (PFIC3), ICP, and gallstone disease and this variant is known to result in reduced MDR3 expression.^[3]

The c.1679C>T (p.Thr560Met) variant is rare and there is less literature on this variant available; however, one case of ICP with a patient heterozygous for this variant has been reported, with in silico predictors of pathogenicity suggesting that this variant is likely to be damaging.^[4]

The patient is also heterozygous for the common 7 promoter repeat in the UGT1A1 gene in keeping with autosomal recessive Gilbert syndrome.

In addition to genetic analysis, the patient had a magnetic resonance cholangiopancreatography which showed no evidence of cholangiopathy, a fibroscan which showed a fibrosis score of 9 kPa and a CAP score of 200 dB/m, and, further, blood tests 4 weeks postnatally which showed normalization of the liver biochemistry and bile acids at which point the patients ursodeoxycholic acid was stopped.

Following her genetic analysis results, her case was discussed with experts in molecular hepatology who believe that the clinical history is in keeping with reduced MDR3 function. Previous experiences with cases with two missense changes have resulted in patients who either only present clinically during pregnancy or those who have proceeded to develop progressive cholangiopathy resulting in the end-stage disease requiring liver transplantation.

Discussion

The ABCB4 gene resides on chromosome 7 (7q21) and encodes the ABCB4/MDR3 protein, which is a P-glycoprotein expressed in the canalicular membrane of hepatocytes.^[5] It has been established that ABCB4 protein defects lead to intrahepatic cholestasis due to the impairment of biliary phospholipids transport resulting in damage to the biliary epithelium and canalicular membrane from the hydrophobic bile salts.^[6]

Around 300 different disease-causing ABCB4 variants have been reported, with the phenotype and severity of liver disease differing in individuals depending on their ABCB4 allelic status. Variations in the ABCB4 gene can be classified as non-sense mutations (class I), missense mutations affecting the maturation (class II), activity (class III), or

stability of the ABCB4/MDR3 protein (class IV), and variations without identifiable effect on protein function and expression (class V).^[7]

ABCB4 mutations have variable clinical presentation and predispose to several human liver diseases. Heterozygotes with functional MDR3 activity may simply manifest with increased serum gamma-glutamyl transferase or a mild chronic cholangiopathy. Those with complete deficiency of MDR3 activity are more likely to get more significant disease.

More than 45 mutations in the ABCB4 gene have been found to cause PFIC3, with affected individuals having mutations in both copies of the ABCB4 gene, leading to liver fibrosis, cirrhosis, and portal hypertension in infancy or childhood.^[8]

Heterozygous or less severe ABCB4 mutations have been associated with low phospholipid-associated cholelithiasis syndrome and also with more severe forms of ICP.^[9,10]

ICP affects 1% of all pregnancies in Europe and much is still unknown regarding the etiology of the disease. Due to increased rates of ICP seen in patients with multiple pregnancies and the rapid cessation of ICP after delivery, it has been hypothesized that the pathogenesis of the disease centers around female sex hormones.^[11] The difference in the incidence of ICP depending on geographic areas and ethnicity highlights the importance of genetic predisposition to this condition. At present, 28 different mutations in the ABCB4 have been associated with ICP.

A recent study performed genetic analysis on 25 patients with ICP and found significant alterations in either; ATP8B1 (n=2), ABCB11 (n=1), or ABCB4 (n=7) genes.^[4] This study also had a patient with the ABCB4 variant c.1679C>T (p.Thr560Met) and is the only study currently published linking this ABCB4 variant with a cholestatic liver disorder. Based on in silico predictors of pathogenicity and the rarity of the mutation, we believe that this patient has reduced MDR3 activity due to two different missense ABCB4 genes and that this has contributed to the patients developing severe recurrent ICP.

Larger molecular genetic studies are required in patients with ICP to allow us to better understand and recognize the potential pathogenic variants in the ABCB4 gene and improve understanding of how this plays a role in the pathogenesis of ICP.

Conclusion

In younger patients with unexplained hepatobiliary disease, particularly those with a family history of liver disease, genetic cholestasis testing should be considered to identify genetic abnormalities affecting transporters in the canalicular membrane of hepatocytes.

Patients with ICP and younger patients with cholestatic drug-induced liver injuries or cholelithiasis may also benefit from genetic testing to look for possible genetic determinants of disease, and larger molecular genetic studies are required in these groups to increase our knowledge and understanding of the various genetic mutations which play a role in these diseases.

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