



BMH Med. J. 2025;12(4):75-80. **Case Report**

Progressive Familial Intrahepatic Cholestasis Due To Mutation Of USP53 Gene

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Abstract

Progressive familial intrahepatic cholestasis (PFIC), first described in 1969, is one of the most important causes of genetic cholestatic liver disease in neonates, infants and older children.

An infant born to non-consanguineous parents presented with cholestatic jaundice. The common causes like biliary atresia, choledochal cyst, hypothyroidism, infections, etc were excluded by relevant investigations. Liver biopsy done later revealed canalicular cholestasis and portal fibrosis. Subsequently, he was lost for treatment for several years as the parents had opted for indigenous treatments. He came back to us 2 years back as a young adult with severe intractable pruritus causing even suicidal thoughts, jaundice and firm hepatomegaly. Clinical exome was suggestive of type 7 progressive familial intrahepatic cholestasis (PFIC7).

Keywords: Neonatal cholestasis, Progressive Familial Intrahepatic Cholestasis, low-GGT, intractable pruritus, USP53 gene

Introduction

Progressive familial intrahepatic cholestasis (PFIC) is a constellation of autosomal recessive diseases involving the hepatocanalicular transporters. These manifest as jaundice, intractable pruritus, and failure to thrive leading to portal hypertension, liver failure, cirrhosis, and hepatocellular carcinoma. Several newer variants of PFIC have been described recently, thanks to the amazing developments in the field of genetics in the last few decades. Current treatment modalities are medical (ursodeoxycholic acid, symptomatic treatment for pruritus, nutritional therapy such as adequate calories, supplementation of fat soluble vitamins and medium chain triglycerides), and surgical (partial biliary diversion and liver transplantation). We are discussing a very rare case of a patient with low-gammaglutamyl transpeptidase cholestasis due to ubiquitin-specific peptidase 53 (USP53) gene mutation (PFIC 7), who first presented initially in neonatal period.

Case Description

A young man born to non-consanguineous parents had presented in neonatal period with jaundice,

pale stools, and choluria. He was the third child out of four, born at term by a normal vaginal delivery with a birth weight of 2900 g. There was no history of intrahepatic cholestasis of pregnancy, or chronic liver disease in the family. Physical examination revealed jaundice and firm hepatomegaly. The common causes of neonatal cholestasis such as biliary atresia, choledochal cyst, hypothyroidism, infections etc were excluded by relevant investigations. His complete blood count and peripheral smear were unremarkable. Total serum bilirubin (TSB) was 9.5 mg/dL, conjugated bilirubin 7.1 mg/dL, AST 110 IU/L, ALT 124 IU/L, ALP 850 IU/L, total protein 6.1 g/dL, albumin 4.1 g/dL, and PT 13 seconds. (GGT was not available during that time). USG was normal except for hepatomegaly. He was given the usual treatment for neonatal cholestatic liver disease, and followed up.

Liver biopsy done during early childhood revealed canalicular cholestasis and portal fibrosis. HBsAg and anti-HCV were negative and markers of autoimmune hepatitis were absent. At school age he had severe pruritus, excoriation marks all over the body, shiny nails, short stature (height 110 cm, < 3rd centile), genu varum, and jaundice. There were no signs of chronic liver disease. His hearing was normal and scholastic performance was poor. Liver was palpable 5 cm below the right costal margin, and was firm and non-tender. There was no splenomegaly or ascites. The possibility of benign recurrent intrahepatic cholestasis was considered. TSB was 3.8 mg/dL, conjugated bilirubin 2.2 mg/dL, AST 140 IU/L, ALT 156 IU/L, ALP 1240 IU/L, total protein 6.5 g/dL, albumin 3.8 g/dL and creatinine 0.9 mg/dL. His GGT was low (10 IU/L- normal 15-132). Serum ceruloplasmin was 30 mg/dL (20-40 mg/dL) and 24 hour urine copper was normal. Ophthalmology evaluation (for posterior embryotoxon and KF ring), and color Doppler echo for congenital heart disease were normal. USG showed increased echotexture of liver with normal gallbladder and bile ducts. He was given two doses of hepatitis A vaccine, in addition to hepatitis B vaccine. He continued to have intolerable pruritus not responding to any treatment. At this juncture his parents opted for indigenous treatment, and hence he was lost for follow up for several years.

He came back to us 2 years ago, with intractable pruritus, fluctuating jaundice, and firm hepatosplenomegaly.

He did not have edema, bleeding or signs of chronic liver disease. TSB was 3.2 mg/dL, conjugated bilirubin 2.1 mg/dL, AST 116 IU/L, ALT 168 IU/L, ALP 950 IU/L, GGT 14 IU/L, total proteins 6.2 g/dL, albumin 3.4 g/dL, PT 14 and INR 1.1. Serum vitamin D was very low (5 ng/ml) and calcium was 7.5 mg/dL. Serum cholesterol was 228 mg/dL, triglycerides 76 mg/dL, HDL 45 mg/dL and LDL 168 mg/dL. USG showed coarse echotexture of liver and mild ascites; gallbladder and bile ducts were normal. Clinical exome sequencing revealed a homozygous variant, likely pathogenic, in intron 11 of the USP53(+) gene (c.822+1del) in the proband, suggestive of PFIC7. (**Figure 1**). Parents and siblings could not be tested for mutation due to financial constraints.

LIKELY PATHOGENIC VARIANT CAUSATIVE OF THE REPORTED PHENOTYPE WAS DETECTED

SNV(s)/INDELS

Gene* (Transcript)	Location	Variant	Zigosity	Disease (OMIM)	Inheritance	Classification
USP53 (+) (ENST00000274030.3)	Intron 11	c.822+1del (5' splice site)	Homozygous	Progressive familial intrahepatic cholestasis-7 with or without hearing loss (OMIM619858)	Autosomal recessive	Likely pathogenic

Figure 1. Clinical exome result

He was treated with vitamin supplements, ursodeoxycholic acid (UDCA), antihistamines (bilastin 20mg/day) and cholestyramine with only partial relief of pruritus. After dermatology consultation he was also given anti-scabies treatment following which his pruritus decreased considerably.

Discussion

Neonatal cholestasis, defined as serum conjugated bilirubin level >1 mg/dL if total serum bilirubin

is < 5 mg/dL, or conjugated bilirubin $> 20\%$ of the total bilirubin if it is > 5 mg/dL, is a relatively common condition that affects approximately one in 2500 infants. Cholestasis is seen in more than 100 hepatobiliary disorders and/or metabolic liver disorders [1].

Bile formation is essential for the normal functioning of liver and the gastrointestinal system. The flow of bile is generated by osmotic forces associated with the secretion of bile salts into biliary canaliculi. Bile secretion from hepatocytes is mediated by a group of transport proteins called ATP-Binding Cassette (ABC) proteins, the most important of which is the bile salt export pump (BSEP), expressed exclusively in the apical/canalicular membrane of hepatocytes.

Our knowledge of the molecular mechanisms of bile secretion has been considerably strengthened by the recent discovery of various genes involved in hereditary intrahepatic cholestasis. Due to the advances in genetic testing and bioinformatics including whole exome or whole genome sequencing, several molecularly defined disorders involving bile formation, canalicular transporters, tight junction proteins and inborn errors of metabolism are being continuously discovered [2].

PFIC is an umbrella term for a group of severe rare heterogeneous genetic disorders with an incidence of about 1 in 50,000 - 100,000 live births, although the exact prevalence is not known. They account for 10 to 15 percent of cases of neonatal cholestasis, and 10 to 15 percent of liver transplants in children. They are caused by mutations of genes of various transporters expressed in the canalicular membrane of hepatocytes or defects of tight junction structure at the level of hepatocytes [3]. They have characteristic clinical, biochemical, and histopathological features. PFIC which is the prototype of genetic liver diseases, manifests as jaundice in the neonatal period/infancy/early childhood. The accumulation of toxic bile acids in hepatocytes leads to severe pruritus and hepatocyte injury ultimately resulting in progressive liver fibrosis/cirrhosis, that may eventually require liver transplantation.

All these disorders are autosomal recessive in inheritance. In one third of patients with a clinical phenotype of PFIC, it may not be possible to identify a known PFIC [4]. The onset and severity of jaundice and cholestasis differ with each type of PFIC.

The first PFIC to be identified was PFIC 1, also known as Greenland familial cholestasis and Byler's disease. It is due to mutation of ATP8B1 (ATPase Phospholipid Transporting 8B1) gene on chromosome 18q21-q22, resulting in deficiency of FIC1 (phosphatidylserine flippase) protein. FIC1 protein helps in the movement of phospholipids such as phosphatidylethanolamine and phosphatidylserine from the outer to the inner canalicular membrane leaflets. The exact mechanism of cholestasis and abnormal bile acid transport due to the mutation of ATP8B1 is still not clear. As ATP8B1 is expressed in several extrahepatic tissues also, PFIC1 is a multisystem disease characterized by recurrent diarrhea, pancreatitis, short stature and sensorineural deafness. Liver biopsy shows bland cholestasis, and electron microscopy characteristic coarse and granular bile (Byler's bile). A condition allelic to PFIC1 is benign recurrent intrahepatic cholestasis type 1 (BRIC1; MIM243300) which is also caused by mutations in ATP8B1.

PFIC 2 (Byler syndrome), the most common PFIC presenting in infancy, is due to mutation of ABCB11 (ATP Binding Cassette Subfamily B Member 11) gene on chromosome 2q24, resulting in deficiency of bile salt export pump (BSEP), also known as sister P-glycoprotein (SPGP). BSEP, identified in 1998, is the major bile acid transporter from hepatocytes into biliary canaliculi. BSEP expression is regulated by Farnesoid X receptor (FXR). When bile acid concentration increases, FXR is activated which upregulates expression of BSEP. This in turn increases bile acid excretion by hepatocytes into the biliary canaliculi. Some of the features of PFIC2 are rapidly progressive cholestatic giant cell hepatitis, growth failure, and intractable pruritus. Liver biopsy shows portal tract fibrosis and bile duct proliferation while electron microscopy reveals amorphous canaliculi.

bile which may help to distinguish PFIC1 from PFIC2. As ABCB11 is expressed only in the liver, extrahepatic manifestations are absent unlike PFIC1. These children have a very high risk of developing hepatocellular carcinoma, cholangiocarcinoma, and gallstones, and hence close monitoring is recommended right from infancy onwards. Following liver transplantation for PFIC2, some children develop recurrence of disease due to autoantibodies against BSEP.

In the subset of patients with ABCB11 missense mutations, pharmacological chaperones such as 4-phenylbutyrate may be beneficial. A milder form of BSEP disease, also caused by mutations in ABCB11, is referred to as benign recurrent intrahepatic cholestasis type 2 (BRIC2).

PFIC 3 due to mutation of ABCB4 (ATP Binding Cassette Subfamily B Member 4) gene located on 7q21, is really a cholangiopathy. It leads to deficiency of the floppase multidrug resistance protein-3 (MDRP-3), which flopps phosphatidylcholine (PC) from the inner to the outer leaflet of the canalicular membrane so that it is secreted into bile, and is then extracted by bile salts to form mixed micelles in the canalicular lumen. The presence of PC in the canaliculi protects cholangiocytes from the deleterious detergent action of toxic bile salts. In its absence i.e. in PFIC3, this protective action is lost. Cholangiocytes which are now damaged by the bile acids secrete copious amounts of GGT. Hence, this is the only PFIC with high serum levels of GGT. It is also the least common type of PFIC to present as neonatal cholestasis but may present at any time during childhood or even in adulthood. These patients have milder pruritus compared to the other forms of PFIC. Portal hypertension, and intraductal and gallbladder lithiasis may be seen. Besides PFIC3, heterozygous carriers of ABCB4 mutations may also develop intrahepatic cholestasis of pregnancy [5]. The concentration of biliary phospholipids may be reduced by heterozygous mutations in ABCB4. This increases the risk for cholesterol stones, microlithiasis, or sludge, a condition known as low phospholipid-associated cholestasis (LPAC).

PFIC 4 is due to protein-truncating mutations of TJP2 (tight junction protein 2, also known as zona-occludens 2) gene. This gene located on 9q21 was discovered in 1991 and is an important cytosolic component of cell-cell junction. Due to the truncation of TJP2 protein it cannot be incorporated in tight junctions. Hence, claudin1 (CLDN1) cannot localize in the cholangiocyte-cholangiocyte borders and margins of bile canaliculi. These patients usually develop severe liver disease early in infancy (1 week to 4 months), and many of them subsequently require liver transplantation. Some of these patients develop respiratory and neurologic manifestations also.

PFIC 5 due to mutation of NR1H4 (Nuclear Receptor Subfamily 1 Group H Member 4) gene located on 12q23 leads to defective FXR which is a nuclear bile acid receptor that regulates BA metabolism. The characteristic histologic features are intralobular cholestasis, ductular reaction and giant cell transformation. It has a very rapid progression, and post-transplant hepatic steatosis is common. PFIC6 is due to mutation of MYO5B (myosin 5b) gene located on 18q21 whose function is cell polarization and trafficking of BSEP. 6 It is characterized by giant cell transformation, hepatocellular and canalicular cholestasis, and a relatively slow progression of disease.

Maddirevula et al recently described 7 families with cholestatic liver diseases who were negative for all known PFIC mutations. Whole exome sequencing led to the identification of 3 new diseases with PFIC-like presentation. These include defects in the USP53 gene (ubiquitin carboxyl-terminal hydrolase 53), LSR gene (Lipolysis stimulated lipoprotein receptor), and WDR83OS gene (WD Repeat Domain 83 Opposite Strand) [2].

USP53 gene belongs to the deubiquitinating enzyme family that interacts with TJP2 in hepatocytes thus helping to maintain cell integrity. Its mutation causes PFIC 7 with or without deafness. Biallelic variants in TJP2 cause deafness and low-GGT intrahepatic cholestasis, with elongated hepatocyte-hepatocyte tight junctions [7]. PFIC 7 is characterized by infantile-onset jaundice and severe pruritus associated with cholestasis, minimally elevated transaminases, high ALP and low/normal GGT. Liver biopsy features include fibrotic changes along with hepatocellular and

canalicular cholestasis. The hepatic abnormalities of many patients with PFIC 7 resolve with age. However, in some patients, severe pruritus, hepatosplenomegaly and elevated liver enzymes persist, as happened in our patient, and some may develop sensorineural deafness. In a study by Zhang et al of 69 infants with low GGT cholestasis, whole exome sequencing identified the presence of biallelic USP53 mutations (homozygous or compound heterozygous) in 7 [8]. A 7-month-old infant with low-GGT PFIC due to novel biallelic mutations in the USP53 gene was described recently. His liver biopsy showed portal and perivenular fibrosis with bland bilirubinostasis [9]. A 2-year-old male child with low GGT cholestasis and multiple hemangiomas of liver was described by Shatokhina et al. Clinical exome revealed a novel homozygous c.1017_1057del (p.(Cys339TrpfsTer7), NM_019050.2) truncating variant in the exon 12 region of the USP53 gene. It is possible that biallelic mutations in the USP53 gene disrupt the formation of dense connections between endothelial cells, which leads to the development of hemangiomas [10].

There have been such tremendous advances in the field of hepatology in the last 50 years that the proportion of children with neonatal cholestasis due to 'neonatal hepatitis' has been drastically reduced from 65% to just 10-12%. Genetic diseases causing cholestasis now account for almost 60% (PFIC ~25%, metabolic disease ~25%, and alpha-1 antitrypsin deficiency ~10%). A biochemical clue for various types of PFIC (except type 3) and inborn errors of bile acid synthesis is low/normal serum GGT level disproportionate to the severity of cholestasis [6].

Some factors that contribute to improved patient care in such diseases are prompt diagnosis and the correct treatment based on genetic studies. The accumulation of excessive amounts of toxic hydrophobic bile acids in the cholestatic hepatocytes resulting in fibrosis and cirrhosis is the final common pathway to various types of PFIC. Thus, one of the therapeutic options is to reduce or prevent the accumulation of toxic bile acids in hepatocytes by drugs such as UDCA [11]. UDCA acts by inducing secretion of bile acids into biliary canaliculi by stimulating BSEP and MRP2, and by diluting the pool of toxic bile acids. Besides, it also has antifibrotic, cytoprotective and anti-inflammatory actions [12]. The possibility of common associated conditions such as scabies that may aggravate pruritus should also be kept in mind in children with cholestasis.

Future therapeutic options in various types of PFIC include mutation targeted pharmacotherapy, gene therapy and hepatocyte transplantation [13].

This patient who first presented first to us at 30 days of life was followed up and a diagnosis of PFIC 7 was confirmed in adulthood.

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