

A novel homozygous mutation in the *USP53* gene as the cause of benign recurrent intrahepatic cholestasis in children: a case report

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ABSTRACT

Background. Benign recurrent intrahepatic cholestasis (BRIC) is a rare cause of cholestasis with recurrent episodes of jaundice and pruritus without extrahepatic bile duct obstruction. A mutation in the *USP53* gene is known to cause BRIC-like cholestasis with normal serum gamma-glutamyltransferase (GGT) levels.

Case. We report a 16-year-old boy with recurrent episodes of cholestasis since 6 months of age with normal serum GGT levels. The liver biopsy showed ballooning degeneration of hepatocytes which is typical for BRIC, and intrahepatic and canalicular cholestasis with bilirubinostasis. We performed whole exome sequencing (WES) and identified a novel homozygous variant (NM_001371399.1:c.1558C>T) of the *USP53* gene at exon 14 as the cause of BRIC.

Conclusion. This is the first case of *USP53* disease from Türkiye with a novel mutation in the *USP53* gene. This novel identification of the mutation of c.1558C>T at exon 14 can provide elucidative data for those who work in the field of intrahepatic cholestasis. Our case suggests that *USP53* disease must be kept in mind in patients with recurrent intrahepatic cholestasis with normal serum GGT levels.

Keywords: cholestasis, children, *USP53* gene, benign recurrent intrahepatic cholestasis (BRIC).

Familial intrahepatic cholestasis (FIC) is a heterogeneous group of liver disorders in which the excretion and production of bile acids from hepatocytes are impaired.¹ Progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC) are considered to be part of a spectrum of intrahepatic cholestasis. The difference between the two diseases is based on phenotypic presentation.² BRIC is an autosomal recessive cholestatic disease characterized by recurrent episodes of cholestasis manifested by jaundice, pruritus, fatigue, and steatorrhea with variable severity and duration.³ Episodes can

be triggered by an infection, and can last from weeks to months.⁴

Genes associated with FIC include *ATP8B1* (FIC1), *ABCB11* (FIC2), *ABCB4* (FIC3), *TJP2* (FIC4), *NR1H4* (FIC5), *SLC51A* (FIC6), *KIF12* (FIC8), *MYO5B* (*MYO5B*-PFIC).^{5,6} Among these, mutations of the *ATP8B1* (BRIC1), *ABCB11* (BRIC2), *TJP2* and *MYO5B* genes are previously defined to be related to BRIC.⁶⁻

⁸ *USP53* (Ubiquitin specific peptidase 53), a member of the deubiquitinating enzyme family, is expressed in the liver, brain, kidney, and inner ear.⁹ As it was previously reported in a mouse-deafness model, *USP53* colocalizes and interacts with tight junction proteins, *TJP1* and *TJP2*, suggesting that *USP53* is a part of the tight junction complex and essential for the stability of tight junctions.¹⁰ *TJP2* gene is known to be associated with a spectrum of cholestatic

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hepatobiliary disease.¹¹ It was preliminarily reported in three Saudi children that asserted the association with the mutation in *USP53* and low-GGT cholestasis in 2019.¹² In addition, in recent studies, the *USP53* disease has been included in the PFIC group, and specified as PFIC type 7.¹³ Here, we report a Turkish child manifesting as BRIC with a novel *USP53* mutation.

Case

A 16-year-old boy was referred to our department with jaundice and pruritus refractory to antihistaminic treatment. He also reported a loss of appetite, dyspepsia, and right upper quadrant pain. His stool was loose and fatty, and he had lost 3 kg in three weeks. He was born to second-degree consanguineous parents. There was no family history of liver disease.

According to his complaints, the jaundice occurred for the first time 6 months ago. After 6 months of follow-up, his symptoms were completely relieved. After symptom-free 9 years, at the age of 10, his parents reported a similar episode characterized by jaundice, pruritus, and loss of appetite, which recovered within a month.

On examination, there was no abdominal tenderness, hepatosplenomegaly, or ascites. Scleral and skin icterus, and scratch marks on his trunk and extremities were noted. Liver enzymes showed cholestatic hepatitis with markedly elevated total and direct bilirubin and mildly elevated aminotransferases; total bilirubin 11.3mg/dl, direct bilirubin 8.9 mg/dl, ALT 56 U/L, AST 46 U/L, gamma-glutamyl transferase (GGT) 15 U/L. Alkaline phosphatase level and international normalized ratio were normal. Total serum bile acids were 242 μ mol/L (reference range; 0-10 μ mol/L). Other biochemical parameters including amylase, lipase, serum electrolytes, and renal function tests were normal. Hepatitis B surface

antigen and antibodies to hepatitis C and A virus were negative. Immunoglobulin G and subtypes including immunoglobulin G4 were normal. Liver-kidney microsomal antibodies, antinuclear antibodies, antimitochondrial antibodies, anti-smooth muscle antibodies were negative and ceruloplasmin, 24-hour urine copper excretion test, and alpha 1 antitrypsin were normal.

Abdominal ultrasonography revealed mild hepatosplenomegaly with grade 1 hepatosteatorosis. Magnetic resonance cholangiopancreatography showed normal intra- and extra-hepatic biliary tree and pancreatic ductal system.

He was prescribed ursodeoxycholic acid, rifampicin, vitamin D, E, and A. Given the progression of the cholestasis (total bilirubin 23.4 mg/dl, direct bilirubin 17.41 mg/dl) and refractory pruritus, endoscopic retrograde cholangiopancreatography (ERCP) was performed; and a nasobiliary drainage catheter and a 5Fr stent were inserted into the pancreatic duct. Remarkable improvement of the cholestasis and pruritus was noted after 48 to 72 hours of nasobiliary drainage catheter insertion. Two days after the ERCP, acute pancreatitis developed with right upper quadrant pain, elevated serum lipase, and amylase levels and heterogeneity of the parenchyma, and increased volume of the pancreas in ultrasonography. During the follow-up he had three episodes of acute pancreatitis in total, one of which was after the ERCP, that resolved with supportive treatment, including fluid resuscitation in the first 48 hours and without any complications.

The liver biopsy showed ballooning degeneration of the hepatocytes, and intrahepatic and canalicular cholestasis with bilirubinostasis without any significant inflammation. There was no evidence of fatty change, portal tract fibrosis, or hepatitis (Fig. 1).

Mutation analysis of cholestasis-related genes, *ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*,

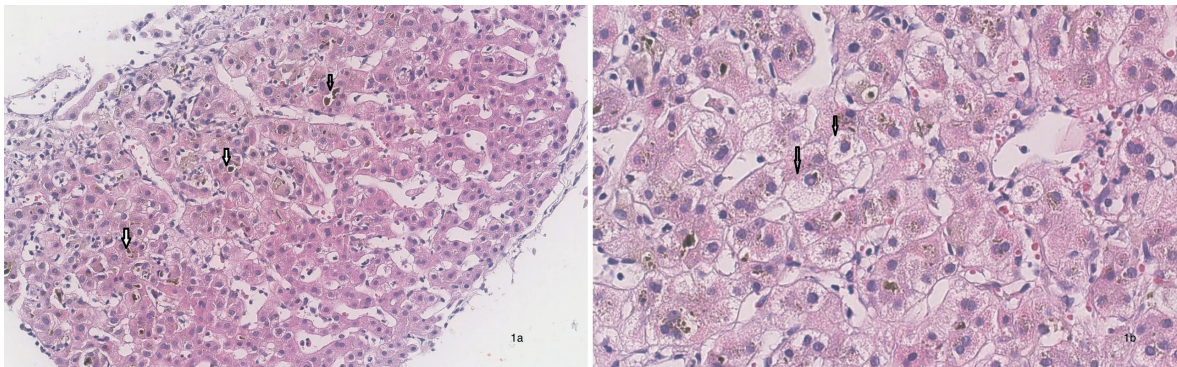


Fig. 1. Histological findings of the patient's liver. Intrahepatic and canalicular cholestasis with bilirubinostasis (1a, arrows), and ballooning degeneration of hepatocytes (1b, arrows). (H&E stain)

NR1H4, *MYO5B* were negative. Whole exome sequencing was performed at Ankara City Hospital, Medical Genetics Laboratory. IDT xGen Exome Research Panel v2 was performed using the Nextseq 550 next-generation sequencing platform (Illumina, San Diego, CA) according to the manufacturer's instructions for the whole-exome sequencing. FASTQ files were analyzed on QCIAU1.6 and the annotation of VCF files was completed by using Qiagen Ingenuity Variant Analysis and Clinical Insight Interpret. For the variant filtering process, we considered only nonsense and missense variants, indels, and variants at canonical splice sites, excluding variants with minor allele frequency greater than 0.01 in different public and local resources. We identified a novel homozygous variant (NM_001371399.1: c.1558C>T) of the *USP53* gene at exon 14. The variant causes premature termination of the protein at p.Arg520Ter. Autosomal recessive inheritance was confirmed by segregation analysis of mutated alleles within parents. We also searched for genes causing recurrent pancreatitis and identified a heterozygous mutation (NM_000492.4: c.3154T>G) on the *CFTR* gene during the WES analysis, which has been implicated in recurrent pancreatitis.

Currently, the patient is still on ursodeoxycholic acid and rifampicin therapy and for 3 months he did not have any cholestatic flares accompanied by jaundice and pruritus. Informed consent was received from the patient and the family.

Discussion

BRIC, which was first described in 1950, is part of a spectrum of familial intrahepatic cholestasis with recurrent episodes of cholestasis.¹⁴ Luketic and Shiffman have since proposed the following diagnostic criteria for BRIC: 1) at least two episodes of jaundice separated by a symptom-free interval lasting several months to years, 2) laboratory data consistent with intrahepatic cholestasis, 3) a normal or minimally-elevated GGT level, 4) severe pruritus secondary to cholestasis, 5) centrilobular cholestasis evident on liver biopsy, 6) normal intra- and extrahepatic bile duct on cholangiography, and 7) an absence of factors known to be associated with cholestasis.¹⁵ Our patient fulfilled these criteria and had been having cholestatic flares since the age of 6 months with a completely asymptomatic period in between.

Mutations in several genes have been reported to cause FIC. Among these *ATP8B1* (BRIC1), *ABCB11* (BRIC2), *TJP2* and *MYO5B* are previously defined mutations known to be related to BRIC.^{2,4,6-8,11,15} By sequencing all coding exons of known cholestasis-related genes, *ATP8B1*, *ABCB11*, *ABCB4*, *TJP2*, *NR1H4*, *MYO5B*, we did not detect any mutation. Because of the typical presentation compatible with BRIC, further investigation was needed and a novel homozygous variant (NM_001371399.1: c.1558C>T) of the *USP53* gene at exon 14 was identified. The relation between a mutation

in the *USP53* gene and cholestasis was first reported in three Saudi children, two sisters and a cousin.¹² All three patients had cholestasis with normal GGT, very high ALP, and hypocalcemia. Unlike the reported cases, there was no confirmed hypocalcemia or elevation in ALP level in any of the cholestatic flares in our patient.

It is reported that USP53 protein gets involved in the tight junction-associated protein family, which maintains the stability of tight junctions and takes part in auditory hair cell and hearing. To date, deafness has been detected in 4 of 22 cases with a known *USP53* mutation.^{9,16,17} As it has been reported that it may develop later in life in patients with normal hearing at the time of diagnosis, our patient is being followed up for possible hearing loss.¹²

Pathological findings of USP53 disease can vary on a patient basis; some of which solely have intrahepatic and canalicular cholestasis, and others have fibrosis with parenchymal nodularity. The liver biopsy of our patient showed ballooning degeneration of hepatocytes, which is typical for BRIC, and intrahepatic and canalicular cholestasis without inflammation, steatosis or fibrosis. Unfortunately, we did not have the opportunity to evaluate the liver tissue on transmission electron microscopy, which could provide more detailed information about typical changes in tight junctions. It is reported that in the tissues of the *USP53*-mutated patients, tight junctions elongate and extend deeper into the paracellular or lateral space, which resembles those in TJP2 disease.¹⁷ Hepatocellular carcinoma may occur in TJP2 disease.^{18,19} Thus it was speculated that USP53 related disease appears not to be entirely benign and patients must be monitored for malignancy.¹⁷

Among the cases described so far, only one patient had liver transplantation. She had a living-related liver transplantation at the age of 6 because of intractable itching that was not responsive to medical treatment.⁹ Our patient

was under traditional medical management with ursodeoxycholic acid and rifampicin. He retains his native liver with normalized bilirubin levels.

Acute recurrent pancreatitis has been previously reported in BRIC patients and this situation has been related to the expression of *ATP8B1*, the responsible gene in BRIC1, in the pancreas.⁴ As a result of genetic analysis to investigate the etiology of recurrent pancreatitis in our patient, we determined a heterozygous mutation in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*). *CFTR* is an ion channel regulating the movement of chloride and bicarbonate across cell membranes. If the *CFTR* gene mutates severely it causes a complete loss of *CFTR* function which results in cystic fibrosis (CF). If the mutation in the *CFTR* gene is selective and only affects the bicarbonate-preferring channel, it does not cause typical CF but has some effects on the pancreas, nasal sinus, and vas deferens in variable degrees.²⁰ Severe, mild-variable, and compound heterozygous *CFTR* mutations are known to be associated with recurrent acute pancreatitis and chronic pancreatitis.^{20,21} Besides these causalities between trans-heterozygous mutations in both *CFTR* and *SPINK1* and pancreatitis had been previously reported.²² The association between pancreatitis and heterozygous mutation in the *CFTR* gene has not been reported yet. And no data has shown that the *USP53* mutation causes pancreatitis as well. Thus, in the patient, the relevance between recurrent pancreatitis, and USP53 disease, and heterozygous mutation in *CFTR* is unclear. In the last instance, the ERCP procedure performed on the patient seems like the most potential cause of the pancreatitis.

Here we report the first case of USP53 related disease from Türkiye. The novel identification of the mutation c.1558C>T at exon 14 can provide elucidative data for those who work in the field of intrahepatic cholestasis. USP53 disease should be considered in patients who present with normal GGT levels and recurrent episodes of cholestasis.

Ethical approval

This study was conducted in adherence to the Declaration of Helsinki and the participation involved informed consent. Informed consent was received from the patient and the family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBA, GH; data collection: FD; analysis and interpretation of results: BBA, ACC, HTD; draft manuscript preparation: BBA, SH. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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