



A novel pathogenic variant of ATP-binding cassette subfamily B member 4 causing gallstones in a young adult

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Abstract

The low phospholipid-associated cholelithiasis (LPAC) syndrome was reported in European adults with cholelithiasis and a mutation of the ATP-binding cassette subfamily B member 4 (*ABCB4*). The *ABCB4* encodes multidrug resistance 3, which is a phospholipid translocator. Reduced phospholipid transport can lead to the formation of biliary cholesterol stones. Here, we describe a 31-year-old Japanese man diagnosed with recurrent biliary colic. Although he recovered quickly after endoscopic treatment for the most recent presentation, he had a family history of similar problems. His mother had required endoscopic treatment for choledocholithiasis and his maternal aunt had died at age 29 years because of liver failure (etiology unknown). We, therefore, performed genetic analysis, which revealed a heterozygous *ABCB4*^{C717S}. LPAC syndrome was diagnosed and the patient has received ursodeoxycholic acid for 2 years with no recurrence. The same variant was identified in the patient's mother, who was subsequently found to have a left intrahepatic calculus requiring left-sided lobectomy. She has received ursodeoxycholic acid for 1 year with no recurrence. *ABCB4*^{C717S} is a novel pathogenic variant, and this is the first patient diagnosed with LPAC syndrome in Japan. We should consider LPAC syndrome in young adults with recurrent cholesterol gallstones to ensure early therapy.

Keywords LPAC · *ABCB4* · MDR3

Introduction

The main risk factors for gallstones are female gender, age, pregnancy, inactivity, and obesity [1]. However, gallstones are rare in young adults without these risk factors. In these cases, multiple and recurrent gallstones can be caused by a mutation in ATP-binding cassette subfamily B member 4 (*ABCB4*) gene [2–5]. The *ABCB4* gene, which encodes multidrug resistance 3 (MDR3), is located on the long arm of chromosome 7 (7q21.12). MDR3 is phospholipid translocator across the hepatocyte canalicular membrane, and

its role in phospholipid translocation is important to their role as a solvent of cholesterol and hydrophobic bile salts in bile by micellization. Reduced or absent phospholipid transport by low- or non-functioning MDR3 can lead to the formation of biliary cholesterol stones and bile salt-induced damage [2–5]. This low phospholipid-associated cholelithiasis (LPAC) syndrome was first described in 2001 [6] as the association of *ABCB4* mutations, low biliary phospholipid concentrations, and recurring symptomatic cholelithiasis. The population prevalence of the disease was estimated to be 1 in 100,000 births in Europe [7]. Thankfully, most patients with LPAC syndrome have favorable outcomes with ursodeoxycholic acid therapy [8]. In this report, we describe the first Japanese case of a patient with LPAC syndrome and report *ABCB4*^{C717S} as a novel pathogenic variant.

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Case report

A 31-year-old man with upper abdominal pain was admitted to our hospital. At ages 18 and 24 years, he had experienced recurrent episodes of upper abdominal pain with liver dysfunction on blood tests and splenomegaly on computed tomography. Clinically, we suspected acute hepatitis at these times, but serological testing showed no abnormalities. When he was 26 years old, based on magnetic resonance cholangiopancreatography showing mild dilatation in some intrahepatic bile ducts, we suspected primary sclerosing cholangitis and performed a liver biopsy. However, the biopsy also showed no abnormality. When he was 30 years old, magnetic resonance cholangiopancreatography was repeated and showed choledocholithiasis, cholecystolithiasis, and intrahepatic stones, so he underwent endoscopic sphincterotomy (Fig. 1a) and laparoscopic cholecystectomy (Fig. 1b). The gallstones were whitish, indicating that they were cholesterol stones. His family history also revealed that his mother had experienced endoscopic treatment for biliary stones at the age of 47 and that his maternal aunt had died of liver failure at the age of 29 years (etiology unknown).

At the current presentation, 6 months after cholecystectomy, the patient reported upper abdominal pain and vomiting. On physical examination, his body temperature was 37.2 °C, blood pressure was 88/37 mmHg, and heart rate was 77 beats per min, while abdominal palpitation revealed epigastric tenderness. He was not obese and did not have an unhealthy diet. Biochemical and hematological investigations indicated the presence of mild inflammation and liver dysfunction: white blood cells, 13,290/ μ L; total bilirubin, 2.7 mg/dL; aspartate aminotransferase, 645 IU/L; alanine aminotransferase, 416 IU/L; lactate dehydrogenase, 475 IU/L; alkaline phosphatase, 484 IU/L; and gamma-glutamyl transpeptidase, 340 IU/L. Contrast-enhanced computed tomography showed that the intrahepatic and common bile ducts were dilated and that both the splenomegaly and the collateral circulation of the portal vein had progressed.

We performed emergency endoscopic retrograde cholangiopancreatography, at which a whitish stone was shown to be impacted at Vater's ampulla (Fig. 2). We removed the impacted stone with a removal balloon and placed an endoscopic naso-biliary drainage tube in the bile duct. He recovered quickly and was discharged on the eighth day of hospitalization. On discharge, we performed genetic analysis with his informed consent (Yamagata University Faculty of Medicine, Institutional Review Board, approval number: H29-395) because of the recurrent gallstones. This revealed a heterozygous *ABCB4* variant, c.T2149A (p.C717S), as shown in Fig. 3. He has since received ursodeoxycholic acid for 2 years and has not suffered recurrence.

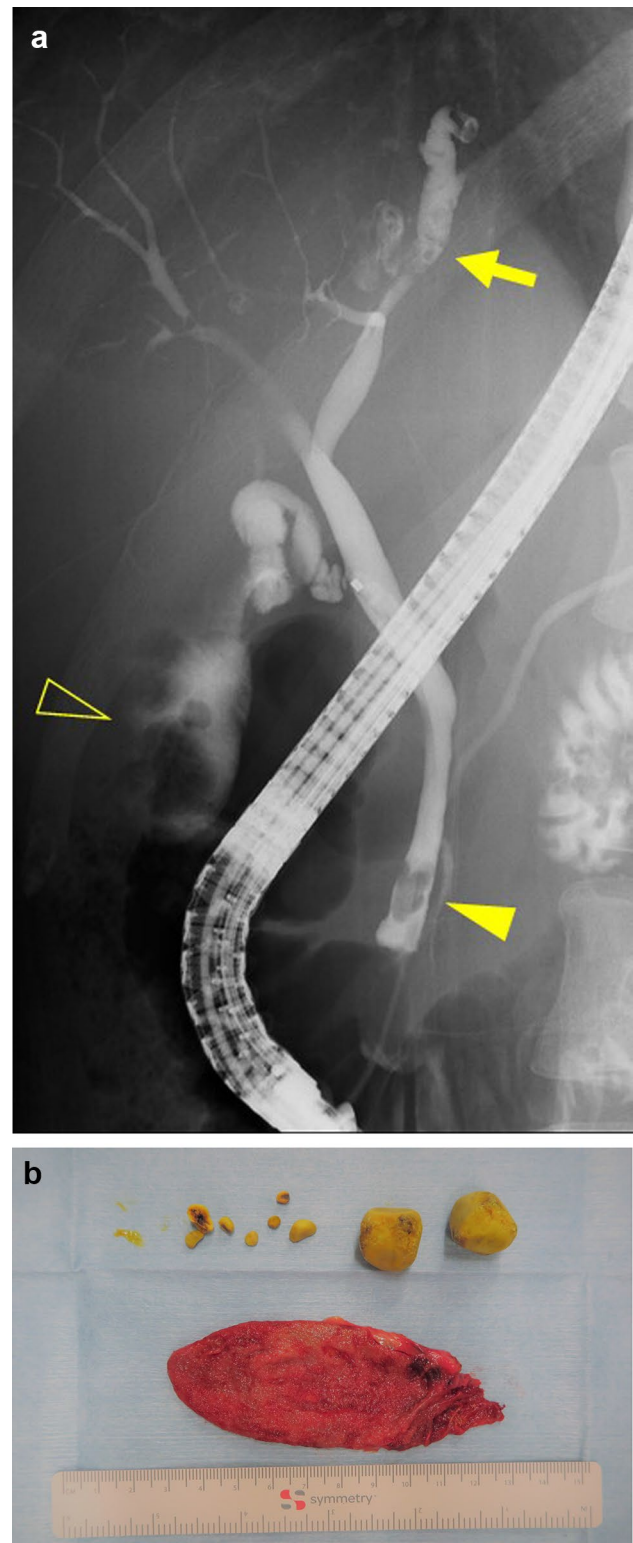


Fig. 1 Cholesterol gallstones seen in the common bile duct and gallbladder. Endoscopic sphincterotomy and laparoscopic cholecystectomy were performed at 30 years of age. **a** Endoscopic retrograde cholangiopancreatography showed choledocholithiasis (closed arrow head), cholecystolithiasis (open arrow head), and intrahepatic stones (closed arrow). **b** Whitish stones in the gallbladder

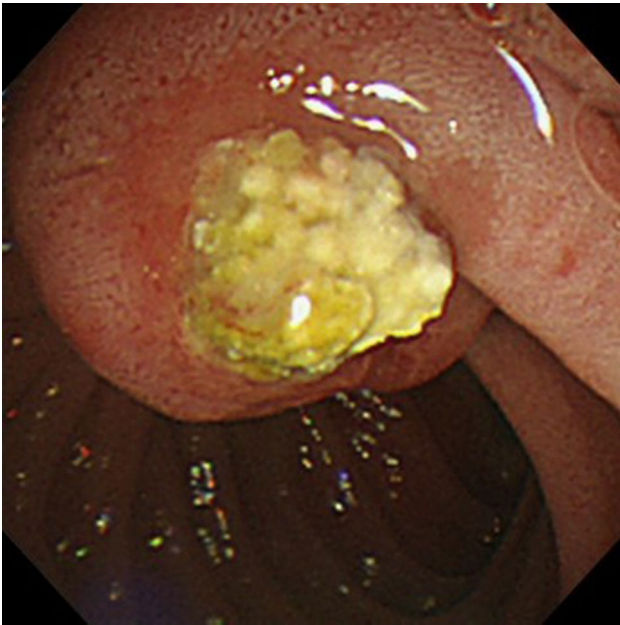


Fig. 2 Urgent endoscopic retrograde cholangiopancreatography. A whitish stone was found, which was impacted at the ampulla

Furthermore, we performed genetic analysis of his immediate family after gaining their informed consent, which revealed the same genetic variant in his mother only (Fig. 3). His mother was a 56-year-old and received a screening test after genetic testing. This uncovered a left intrahepatic calculus and liver abscess for which she underwent a left lobectomy. Pathologically, she had marked fibrosis in the portal region, but no cancer cells were observed. She has since received ursodeoxycholic acid for 1 year without recurrence.

Method used for the mutation analysis

Genomic DNA was extracted from the peripheral blood of each subject using a DNeasy Blood. Whole-exome sequencing (WES) was performed using amplicon-based, next-generation sequencing. Briefly, amplified 317,368 amplicon libraries of 155–245 bp size were constructed using an Ion AmpliSeq Library Kit v2.0 (Thermo Fisher Scientific, Carlsbad, CA, USA), according to the manufacturer's instructions. After adaptor ligation reaction, quantification of the libraries was performed on a 2200 TapeStation Instrument using D1000 Reagents and D1000 Screen Tape (Agilent Technologies, Santa Clara, CA, USA). Each 100 pmol of the amplified libraries was submitted to emulsion polymerase chain reaction using an Ion OneTouch2 Instrument with an Ion P1 Template Hi-Q OT2 200 Kit. Ion sphere particles were enriched using the Ion OneTouch ES Instrument. Ion sphere particles were then loaded on an Ion P1 Chip. Massive parallel sequencing was performed by an Ion Proton

semi-conductor sequencer with an Ion P1 Hi-Q Sequencing 200 Kit.

Read sequence files were assessed through two independent genetic variant calling pipelines, Bowtie2-Platypus and BWA-Platypus, to address the human reference assembly (hg38) and to obtain the variant call format files. Then, concordant genetic variants were annotated using ANNOVAR, with population technical frequency from 1000 Genome and ExAC databases, protein damaging Phred score from SHIFT, GERP, and CADD databases, and genetic pathogenicity from the ClinVar database.

Finally, to confirm the mutation identified by WES, Sanger sequencing was performed using a BigDye V3.1 terminator Kit on ABI 3500 sequencer (Thermo Fisher Scientific). Primer sequences were obtained from Primer3. Polymerase chain reaction was performed to obtain a 680-bp product for the targeting chromosome position of *ABCB4* exon 17. Sequence data were analyzed using EMBOSS and BLAST.

Discussion

The clinical phenotypes of *ABCB4* mutations are broad but can broadly be divided into two forms. On the one hand, missense and heterozygous forms tend to be associated with biliary stone formation in young adulthood. On the other hand, nonsense and homozygous forms tend to progress to liver cirrhosis in childhood [9]. Furthermore, several studies have reported that mutations in *ABCB4* increased the risk of cholangiocarcinoma, hepatocellular carcinoma, and gallbladder carcinoma [10–16]. *ABCB4* mutation-associated cholelithiasis in young adults has been named the LPAC syndrome [6], and these patients typically present younger than 40 years at symptom onset and with recurrent biliary symptoms after cholecystectomy. Splenomegaly was observed in our case, which seems to have been caused by liver damage due to *MDR3* dysfunction.

Most cases of LPAC syndrome are of the heterozygous missense form [8], with most disease-causing mutations localized to the central part of the molecule, close to nucleotide-binding domain 1, or in adjacent transmembrane domains [9]. Transmembrane 7, encoded by exon 17, is a part of the phospholipid translocation pathway [17]. To date, the *C717S* of *ABCB4* detected in our patient and his mother has not been described. This variant located in exon 17 leads to a substitution of the amino acid serine with the amino acid cysteine at position 717, causing a neutral residue to be substituted with a polar residue. In turn, this may alter the interaction of the transmembrane segment of the phospholipid-binding pocket with phospholipids. The *C717S* of *ABCB4* is conserved in mammalian *ABCB4* and human *ABCB1*. ExAC, which is the largest reference dataset

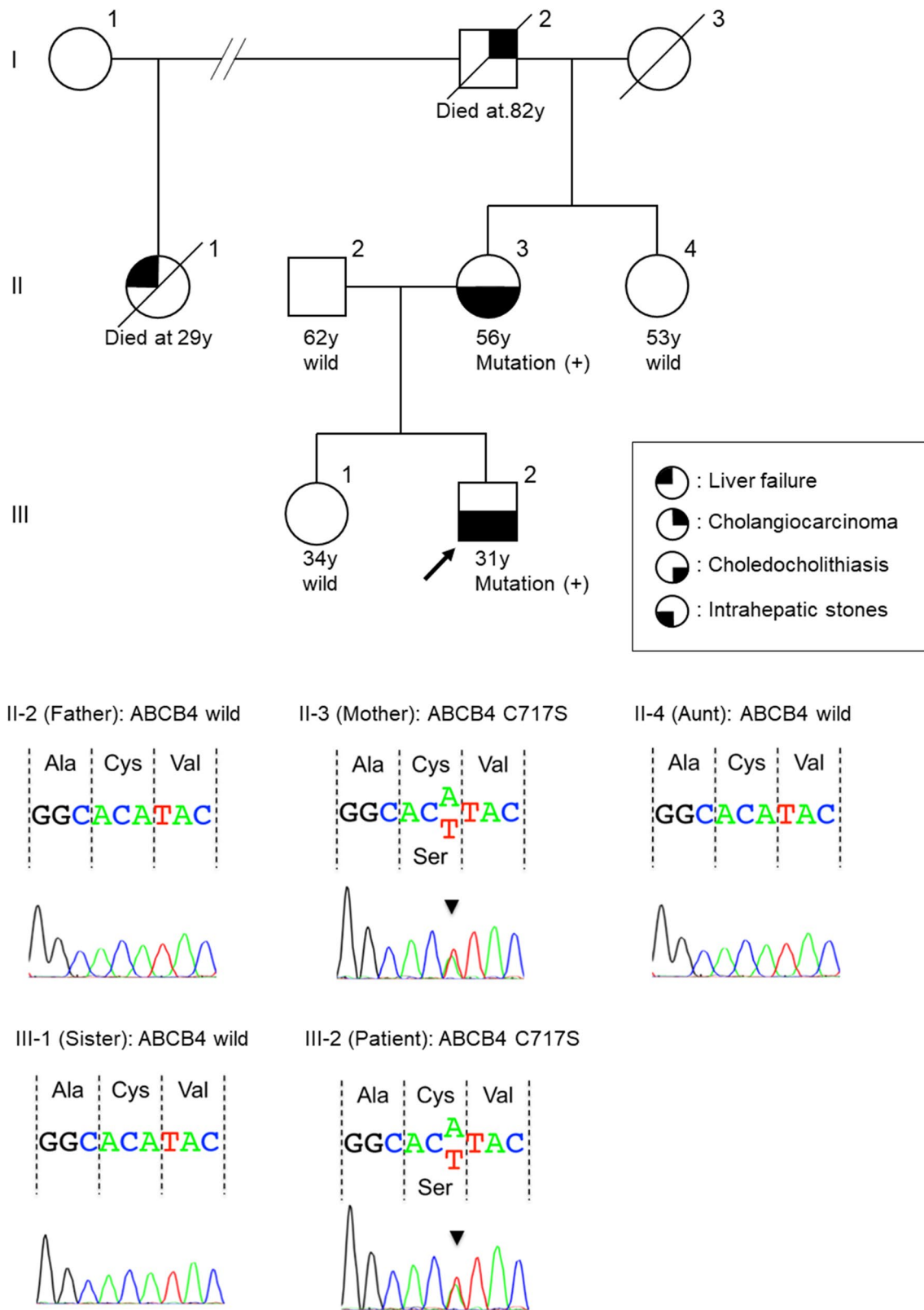


Fig. 3 Genetic analysis. Direct sequencing revealed a heterozygous mutation (c.T2149A in the *ABCB4* gene) in the patient and his mother

of human genetic variation [18], indicates that the frequency of C717S in *ABCB4* is 0.000008239, making it exceedingly rare. We validated the same variant in the patient's mother, who had also suffered from gallstones. Furthermore, the C717S in *ABCB4* was predicted to be probably damaged by GERP++ [19]. Accordingly, we deemed that the C717S in *ABCB4* caused disease.

Treat-to-target in patients with LPAC syndrome aims to prevent gallstone recurrence and progressive liver damage. For this purpose, ursodeoxycholic acid therapy has been shown to be effective in most patients [9]. Ursodeoxycholic acid is hypothesized to increase hydrophilic bile acid and to stimulate biliary secretion [20]. Recently, the cystic fibrosis transmembrane conductance regulator potentiator, ivacaftor, was reported to be a potential therapy in selected patients who harbor mutations in the ATP-binding site of *ABCB4* [21].

In conclusion, *ABCB4*^{C717S} was identified as a novel disease-causing variant in this first case report of a Japanese patient with LPAC syndrome. This diagnosis should be considered in young adults with recurrent cholesterol gallstones, splenomegaly, and a family history of relevant disease. When diagnosed, therapy with ursodeoxycholic acid should be initiated early to prevent recurrent gallstones and progressive liver damage.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Human/animal rights All procedures followed have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Informed consent was obtained from all patients for being included in the study.

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