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Brief Report

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A novel *ABCB11* variant in compound heterozygosity: BRIC2 or PFIC2?

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Introduction

The *ABCB11* gene encodes for the bile salt export pump (BSEP)—the main exporter of bile acids expressed on the canalicular membrane of the hepatocyte [1–3]. Pathogenic variants in the *ABCB11* gene can lead to a range of clinical conditions. These conditions vary from benign recurrent intrahepatic cholestasis type 2 (BRIC2), which is a non-severe disorder characterized by occasional episodes of low levels of gamma-glutamyl transferase (GGT) cholestasis, to progressive familial intrahepatic cholestasis type 2 (PFIC2), which is more severe and may necessitate liver transplantation. Recently, odevixibat—an inhibitor of the ileal bile acid transporter—was able to reduce itching and serum bile acid concentrations in a randomized–controlled study, including patients with PFIC2. Odevixibat provide treatment benefits in a disease with high unmet medical needs [4].

Case report

A 17-year-old woman was admitted to our Liver Unit because of severe jaundice, itching with scratching hematomas, and weakness experienced over the previous 2 months. During that same time, she also reported experiencing amenorrhea, diarrhea, and a weight loss of ∼10 kg. One week before the onset of itching, the patient had a fever and cough, for which she was treated with clarithromycin and paracetamol with clinical benefits. She reported another episode of itching dating back to 2 years earlier. At this time, she did not experience any jaundice and went into spontaneous complete remission in 1 month. As per her family history, her mother and a maternal aunt underwent cholecystectomy for cholelithiasis at the ages of 20 and 30 years, respectively.

Laboratory tests upon admission showed total bilirubin 25.8 mg/dL, direct bilirubin 23.5 mg/dL, aspartate transaminase 121 UI/L, alanine transaminase 125 UI/L, alkaline phosphatase 334 UI/L, GGT 19 UI/L, amylase 101 UI/L, serum iron 265 µg/dL, triglycerides 286 mg/dL, total cholesterol 170 mg/dL, creatinine 0.77 mg/dL, sodium 141 mmol/L, potassium 5 mmol/L, albumin 4.2 g/dL, hemoglobin 11.3 g/dL, white blood cell count 1.242×10^9/L, platelets 444×10^9/L, international normalized ratio 0.97, partial thromboplastin time 32.5 s, fibrinogen 747 mg/dL, and serum bile acid 99.8 mmol/L.

Viral hepatitis A, B, C, and E antibodies and molecular research for intestinal pathogens (virus, bacteria, and parasites) on stool sample were negative. IgM and IgG antibodies against Epstein–Barr virus (EBV) were positive, but EBV-DNA was undetectable. Anti-mitochondrial, anti-nuclear, anti-smooth muscle, anti-liver/kidney microsome-1, and anti-transglutaminase antibodies were negative. Immunoglobulins and IgG subclasses were normal. MRI of the abdomen ruled out biliary obstruction or cholelithiasis. Endoscopic ultrasonography confirmed the absence of biliary tract dilatation and anatomical obstruction. She was treated with ursodeoxycholic acid, colestyramine, and cetirizine, with the disappearance of jaundice and itching, and progressive improvement in liver function tests.

Liver biopsy showed minimal inflammatory infiltrate, including some eosinophilic and neutrophilic granulocytes, with mild portal and pericellular fibrosis; in the hepatic lobules, there was widespread canalicular, hepatocellular cholestasis that engaged also Kupffer cells with cholestatic rosettes and some acidophilic bodies with a few outbreaks of inflammatory activity (Figure 1A and B). Immunohistochemical staining showed a normal expression of multidrug resistance protein 3 (MDR3) and a very weak expression of BSEP (Figure 1C–F). These findings were indicative of BSEP-related cholestasis. Genetic testing was scheduled, and the patient was discharged with ongoing symptomatic therapy and an outpatient follow-up program.

WES-based multigene panel associated with hyperbilirubinemia (84 genes) was performed using the Whole Exome Solutions kit by Sophia Genetics. DNA from peripheral blood samples from the proband and her parents were isolated and subjected to

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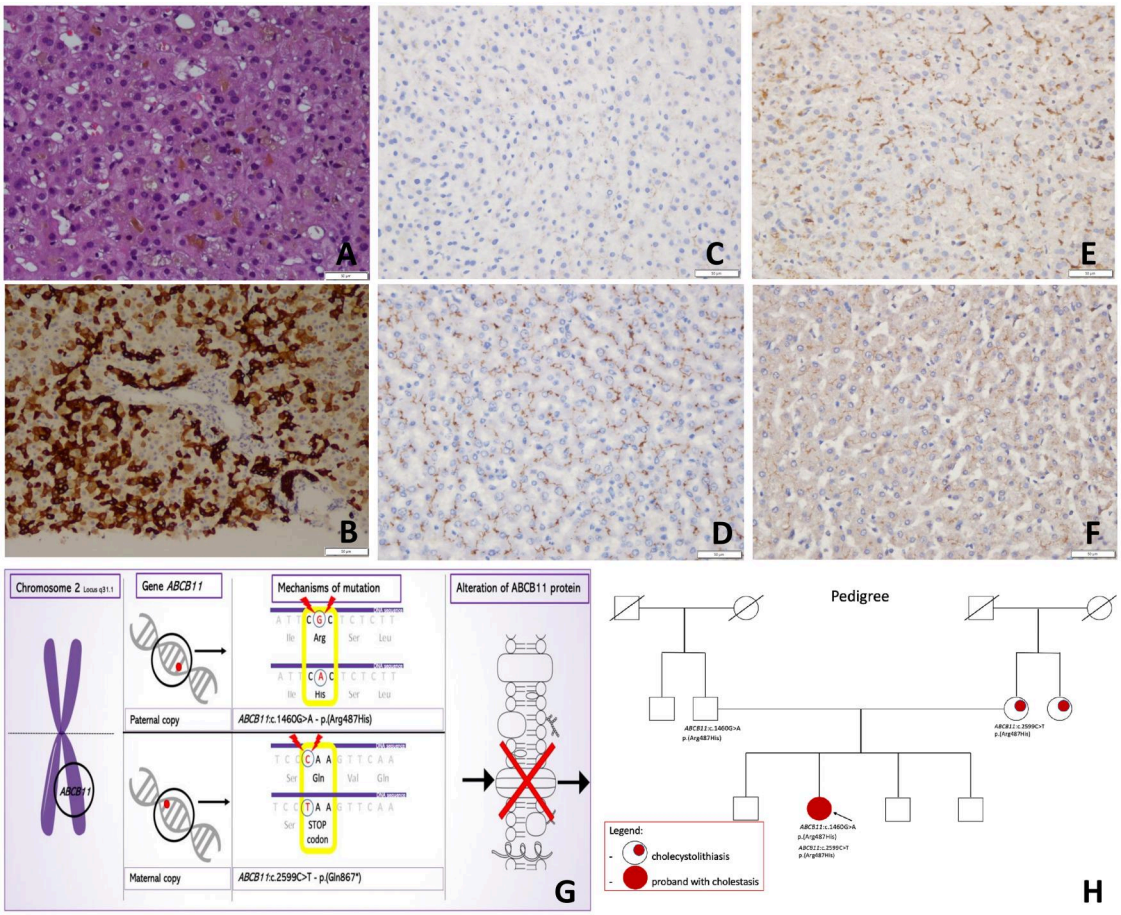

Figure 1. Liver biopsy, genetic infographic, and family pedigree. (A) Liver biopsy (all images 40×). Hematoxylin-eosin staining shows in the portal spaces a minimal mixed inflammatory infiltrate including some eosinophilic and neutrophilic granulocytes with mild portal and pericellular fibrosis; in the hepatic lobule, there was a widespread canalicular, hepatocellular cholestasis that engaged also Kupffer cells with cholestatic rosettes and some acidophilic bodies with a few outbreaks of inflammatory activity. (B) Cytokeratin 7 staining shows intense and diffuse biliary metaplasia of hepatocytes and the presence of bile ducts in portal spaces. (C) BSEP staining shows diffuse low expression. (D) BSEP staining in control liver. (E) MDR3 staining shows normal expression. (F) MDR3 staining in control liver. (G) Schematic representation of the compound heterozygosity status for ABCB11 variants in our proband. (H) Family pedigree. Segregation of the variants in the *ABCB11* gene is shown. The c.2599C>T variant was detected in the mother and the c.1460G>A variant was detected in the father.

exome sequencing using the Clinical Exome Solution Panel by Sophia (Sophia Genetics, SA) on a MiSeq platform (Illumina, San Diego, CA). The software Sequencing Analysis SOPHIA DDM v5.10.29 was used for mutation detection. The analysis examined genes at ≥20× coverage that could provide 99% sensitivity. The analysis showed compound heterozygosity for two variants in the *ABCB11* gene: c.1460G>A–p.(Arg487His) and c.2599C>T–p.(Gln867*). Segregation analysis revealed biparental inheritance of the variants: the father and mother were heterozygotes for c.1460G>A and c.2599C>T, respectively. Sanger sequencing has confirmed the presence of the *ABCB11* mutations (Figure 1G and H).

After 4 months, liver function test levels came back normal and treatment was withdrawn. At her 1-year follow-up visit, the patient was found to be doing well, having experienced no further episodes of jaundice or itching.

Discussion

Here, we reported on a teenager who presented an episode of jaundice and cholestasis associated with a biallelic pathogenic variant in the *ABCB11* gene that has not previously been described.

The c.1460G>A variant is a missense mutation present in the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/variant/2-169828535-C-T?dataset=gnomad_r2_1) with an allelic frequency of 0.000161 in individuals of European non-Finnish origin and in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/290081/) with conflicting interpretations of pathogenicity (three pathogenic, two likely pathogenic, and one of uncertain significance) [5, 6]. *In silico* studies suggest that the c.1460G>A variant may disrupt ABCB11 protein function [6]. Furthermore, as in our case, the variant has been detected *in trans* with other *ABCB11* pathogenic variants in different patients with a diagnosis of PFIC2 [7], BRIC2 [8], and intermediate forms in the spectrum of BSEP-related disorders [9] while no homozygote for c.1460G>A has been described so far. Other missense variants affecting the same amino acid residue, namely p.(Arg487Pro), p.(Arg487Cys), and p.(Arg487Leu), have been classified as pathogenic or likely pathogenic: in particular, p.(Arg487Pro) has been detected in the homozygous state in a patient with severe manifestations [10]. Overall, these data allowed us to classify the variant as likely pathogenic (class IV), in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines [11].

To the best of our knowledge, the c.2599C>T–p.(Gln867*) variant has not been reported so far in the literature and is not listed in ClinVar and gnomAD. This sequence change replaces glutamine at codon 867 of the BSEP protein with a stop codon, leading to premature protein truncation. Therefore, this variant can be classified as likely pathogenic (class IV) according to the ACMG guidelines [11]. Studies attempting to establish genotype–phenotype correlations have also been undertaken. Loss-of-function variants in *ABCB11* are known to be pathogenic and associated mainly with PFIC2 [3, 10] and the most severe presentations have been related to mutations leading to a truncated or non-functional protein compared with missense mutations [12]. It should be noted that other pathogenic variants leading to protein truncation involving the same exon containing c.2599C>T have been classified as pathogenic. In particular, compound heterozygosity for the c.2488del-p.(Arg830fs) and an *ABCB11* missense variant (ClinVar variation ID: 1028652) has been previously reported in association with BRIC2 [8].

The clinical picture of our proband, characterized by late age of onset and benign course with complete recovery, made a diagnosis of BRIC2 more probable. However, the patient requires follow-up over time, especially in the case of pregnancy, to verify the clinical course.

The anti-BSEP antibody used for immunohistochemistry in our laboratory recognizes the 630–750 sequence preceding Gln867. Hence, the absence of BSEP expression in the pathology exam confirms that the truncated protein was absent. In BRIC2 disease, which is characterized by intermittent episodes of intrahepatic cholestasis followed by asymptomatic intervals, the histological staining exam usually reveals, during the flare-up, an almost complete absence of BSEP from the canalicular membrane of liver cells, while BSEP expression returns to normal between episodes [13].

The so-far benign course of the disease in our patient, characterized by a first episode of itching at 15 years old and a second episode of jaundice and itching at 17 years old, both with complete resolution, ultimately led to the decision not to start treatment with odeviximab. This, however, may be subject to reconsideration in the event of a recurrence of cholestasis.

Ethical statement

The patient has provided informed consent for publication of the case and the study received acknowledgement from the Internal Review Board of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome.

Authors' Contributions

R.T. and N.V. collected the data. M.C.G. and P.F. performed pathological staining. A.G. A.B., E.F., and M.G. performed genetic analysis. M.B. drafted the manuscript. G.M., L.M., and A.G. edited and revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

A.G. received a grant from Albireo Pharma Inc. The remaining authors have no conflicts of interest to disclose.

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