Common *ABCB4* and *ABCB11* Genotypes Are Associated with Idiopathic Chronic Cholestasis in Adults

Christoph Jüngst^{a, b} Christina Justinger^a Janett Fischer^c Thomas Berg^c
Frank Lammert^{a, d}

^aDepartment of Medicine II, Saarland University Medical Center, Saarland University, Homburg, Germany;

^bDepartment of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland; ^cDivision of Hepatology, Department of Medicine II, Leipzig University Medical Center, Leipzig, Germany; ^dHannover Medical School (MHH), Hannover, Germany

Keywords

ATP-binding cassette transporter · Cholangiopathy · Chronic liver disease · Genetic risk factor

Abstract

Introduction: Pathogenic mutations in genes encoding the hepatocanalicular transporters *ATP8B1*, *ABCB11* and *ABCB4* are causative for progressive cholestatic liver disease in children. In adults, less severe variants such as the common *ABCB4* c.711A>T polymorphism have been associated with intrahepatic cholestasis in pregnancy and elevated liver enzymes. Hence, our aim was to study the role of common polymorphisms in adult patients with chronic unexplained cholestasis. **Methods:** Screening of outpatients of two university hospitals identified a cohort of 94 patients with chronic cholestasis of unknown origin after thorough exclusion of other causes. Genotyping was performed using Taq-Man assays, and frequencies for the *ABCB4* rs2109505 (c.711A>T), rs1202283 (c.504T>C), *ABCB11* rs2287622 (p.A444V) and rs497692 (c.3084A>G) variants of the study cohort were compared to a cohort of 254 healthy controls. **Results:** The dominating symptoms of the patients were pruritus and jaundice, though the majority of them did not report

symptoms at inclusion. Advanced fibrosis or cirrhosis was present in 11 patients (11.7%) only. Genotyping revealed the presence of the *ABCB4* c.711A>T risk variant in 79 patients (84%), a frequency that is significantly ($p = 0.037$) higher than that in controls (71%). The *ABCB11* p.A444V variant was also more frequent in cholestatic patients ($p = 0.042$). **Conclusion:** The common *ABCB4* c.711A>T and *ABCB11* p.A444V polymorphisms are more prevalent in adult patients with idiopathic cholestasis than in healthy controls and may therefore represent risk factors for the development of chronic cholestatic liver disease.

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Introduction

Chronic cholestatic liver diseases comprise a spectrum of different etiologies such as primary biliary cholangitis (PBC), primary sclerosing cholangitis, secondary sclerosing cholangitis, and IgG4 cholangiopathy. In cholestatic patients, a stepwise diagnostic approach of serological and

The senior authors Thomas Berg and Frank Lammert contributed equally.

imaging studies is recommended, including genetic testing [1–3]. Hereditary cholestasis is well-characterized in pediatric patients, where an important entity represents progressive familial intrahepatic cholestasis (PFIC), which refers to a clinically heterogeneous group of autosomal-recessive diseases due to disturbed excretion of bile and intrahepatic cholestasis [4–8]. The 3 major PFIC types result from mutations in the hepatocanalicular transporter genes *ATP8B1* (PFIC1), *ABCB11* (PFIC2), and *ABCB4* (PFIC3), respectively. *ATP8B1* encodes a phosphatidylserine flippase, *ABCB11* represents the major bile salt export pump, and *ABCB4* encodes a phospholipid transporter responsible for the secretion of phosphatidylcholine into bile. PFIC typically manifests in the first years of life with pruritus and jaundice and may rapidly progress to cirrhosis, often leading to liver transplantation at the age of 3–15 years [9, 10]. Only recently, another 3 types of PFIC (4–6) in distinct genes affecting tight junctions, intracellular signaling, or trafficking have been identified [4, 8, 11].

In adults, intermittent cholestatic episodes with jaundice, mostly resolving without progression to chronic liver disease, have been attributed to variants in the *ATP8B1* and *ABCB11* genes and designated as benign recurrent intrahepatic cholestasis [12, 13]. More recently, it appeared that also conditions of chronic cholestasis may have a genetic basis since mutations in the *ABCB4* gene have been described in adult patients with chronic unexplained cholestasis, which may even progress to advanced fibrosis and cirrhosis [10, 14–16]. Beside disease-causing mutations, also common single nucleotide polymorphisms (SNPs) which are gene variants with a minor allele frequency of >1% have been associated with cholestatic conditions such as intrahepatic cholestasis of pregnancy (ICP) [17–19]. In a large-scale whole-genome sequencing study in the population of Iceland, Gudbjartsson et al. [20] showed that not only rare high-impact *ABCB4* variants are associated with early onset of gallstone disease, ICP, cirrhosis, and hepatobiliary cancer but reported that the common SNP *ABCB4* c.711A>T is a risk factor for elevated aminotransferase and γ -glutamyltransferase (GGT) activities [20]. This study in the Icelandic population suggests that *ABCB4* variants may represent a general risk factor for liver disease.

The *ABCB4* c.711A>T variant may also be of relevance in other populations and play a role, next to rare disease-causing mutations, in patients with unexplained cholestasis. Therefore, we studied common variants in hepatocanalicular transporter genes in a phenotypically well-characterized real-life cohort of adult patients from Germany with chronic idiopathic cholestasis in comparison with healthy controls.

Patients and Methods

Patients

Adult patients with idiopathic cholestasis presenting at the Department of Medicine II, Saarland University Medical Center, Homburg, and the Clinic of Gastroenterology, University Hospital Leipzig, Germany, between 2009 and 2018 were evaluated for inclusion in the study. Idiopathic cholestasis was defined as increased serum alkaline phosphatase (AP) and/or GGT activities of >1.5 times the upper limit of normal for >6 months, without signs of obstructive biliary disease in imaging and no evidence of other chronic liver diseases. These were excluded by comprehensive serology studies comprising hepatitis B, C, D, or E virus infections, ferritin, transferrin saturation, ceruloplasmin, and autoantibodies (anti-mitochondrial type M2, antinuclear, anti-smooth muscle, anti-liver kidney microsomal, anti-soluble liver antigen/liver pancreas, and perinuclear anti-neutrophilic cytoplasmic antibodies). Patients with alcohol-associated liver disease or metabolic-associated fatty liver disease were not included. Patients presenting with the low phospholipid-associated cholelithiasis syndrome (LPAC), according to the standard definition [21, 22], were not included either. In particular, patients harboring pathogenic variants associated with benign recurrent intrahepatic cholestasis or PFIC in *ATP8B1* (variant *rs146599962* [p.N45T], *rs34018205* [p.E429A], *rs121909100* [p.I661T]), or *ABCB11* (variant *rs11568372* [p.E297G] and *rs72549402* [p.D482G]) were excluded from the study.

Clinical data were collected by chart review, including clinical symptoms such as jaundice and pruritus, history of liver disease, diagnosis of cirrhosis, family history of liver disease, gallstone disease, history of ICP, medical therapy, particularly treatment with ursodeoxycholic acid (UDCA), diabetes, metabolic syndrome, and alcohol consumption. In addition, questionnaires were sent to the patients including questions concerning clinical symptoms, in particular pruritus and jaundice, history of liver disease, family history of liver disease, diabetes, alcohol consumption (symptomatic) gallstone disease and cholecystectomy, medical therapy with UDCA as well as symptoms or complications indicating ICP. Follow-up information was compiled when available to analyze the outcome of the patients.

Laboratory results were acquired by chart review. Values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT, AP, and total serum bilirubin most closely to the date of the genetic analysis were identified. Transient elastography by Fibroscan[®] was performed for noninvasive assessment of liver fibrosis in 60 patients (58.8%), as described previously [23, 24]. Since cutoff values for different stages of liver fibrosis have not been evaluated for patients with chronic unexplained cholestasis, thresholds suggested for patients with primary biliary cholangitis were used for staging of fibrosis [25]. Patients with liver stiffness measurements (LSM) ≥ 10.7 kPa were considered to have developed at least advanced fibrosis ($\geq F3$), and the cutoff for cirrhosis was ≥ 16.9 kPa. As a control group for genetic analysis, blood samples of 254 healthy Caucasian blood donors were collected at the Institute of Transfusion Medicine and Immunology, Heidelberg University, Medical Faculty Mannheim, German Red Cross Blood Service Baden-Württemberg – Hessen, Mannheim, Germany. The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Ärztekammer des Saarlandes (271/11) and Leipzig (033/18-ek). All patients in the study provided informed consent.

Genotyping

Genomic DNA from EDTA-anticoagulated blood was extracted using a membrane-based extraction kit (Qiagen, Hilden, Germany). 5'-nuclease PCR based assays with allele specific fluorescent probes (Fisher Scientific, Schwerte, Germany) on a TaqMan® Real Time PCR Fast 7500 system (Applied Biosystems, Foster City, CA, USA) was used for genotyping the hepatocellular transporter gene variants *ABCB4* rs1202283 (c.504T>C), rs2109505 (c.711A>T), *ABCB11* rs2287622 (p.A444V), rs497692 (c.3084A>G), rs72549402 (p.D482G), and *ATP8B1* rs121909100 (p.I661T). The following self-designed primers and probes were used for genotyping the *ATP8B1* variant rs34018205 (p.E429A): forward TCAACTGGGACCTGCAAAATGT, VIC-TGTCCTTCTCAGCATAGT, and FAM-CCTTCGCAGCATAGT. XXA 10 µL PCR reactions contained 20 ng genomic DNA, 1× TaqMan GTXpress Master Mix (Applied Biosystems), 900 nM of each primer, and 200 nM of VIC-labeled and FAM-labeled probes, respectively. Amplification conditions were as follows: 95°C for 20 s, followed by 40 cycles of 95°C for 3 s, and 60°C for 30 s. Direct sequencing was performed for genotyping of the *ABCB11* rs11568372 (p.E297G) and *ATP8B1* rs146599962 (p.N45T) variants using the Big Dye® Terminator 1.1 sequencing kit (Applied Biosystems) of 268 and 295 bp amplicons, respectively, containing the mutations of interest.

Statistical Analysis

Quantitative variables were expressed as median and range, and data analysis was performed by Fisher's exact or Mann-Whitney tests, respectively. For data collection, Excel 2007 (Microsoft, Redmond, WA, USA) was used. Genotyping results of hepatocellular transporter gene polymorphisms were tested for consistency with the Hardy-Weinberg equilibrium by exact tests. Differences of allele and genotype frequencies were assessed by χ^2 and Armitage's trend tests, respectively (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). A *p* value of <0.05 was considered statistically significant.

Results

Patients Characteristics

In total, 94 patients were included in the study population. Of the initially diagnosed 107 patients with idiopathic cholestasis, 13 were excluded after genotyping revealed one of the tested pathogenic mutations in *ATP8B1* or *ABCB11*. Table 1 shows detailed clinical and laboratory characteristics of the entire cohort. In total, 56 (59.6%) patients were women, and the median age at the time of genotyping was 46 years (range 15–78). There were no relatives among the 94 patients included in the study.

Clinically jaundice was present in 19 of 75 patients (25%) with available data, pruritus in 31 patients (41%), and 42 patients did not present symptoms at inclusion (56%). A history of biliary symptoms was reported by 15 of 88 patients (17%), and cholecystectomy had been per-

Table 1. Clinical und laboratory characteristics of 94 patients with unexplained cholestasis

Parameter	N
Age, years	46 (15–78)
Gender	56 F/38 M
Clinical characteristics, n (%)	
Pruritus	31 (41.3)
Jaundice	19 (25.3)
Biliary symptoms	15 (17.0)
History of ICP	5 (17.2)
Advanced fibrosis (≥F3/F4)	11 (11.7)
Cirrhosis	5 (5.3)
Laboratory values	
GGT, U/L	175 (9–2567)
Alkaline phosphatase, U/L	130 (37–1147)
ALT, U/L	69 (14–1443)
AST, U/L	40 (19–556)
Bilirubin, mg/dL	0.5 (0.2–45)

Values are given as median and range. Percentage is given in relation to number of patients with available data on specific parameter. ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; GGT, gamma-glutamyltransferase; ICP, intrahepatic cholestasis of pregnancy; M, male.

formed in 13 patients (15%). Of the 29 patients with previous pregnancies, 5 patients had a history of ICP. A family history of liver disease was reported by 17 of 57 (30%) patients with available data.

Table 1 summarizes the laboratory results of GGT, AP, ALT, AST, and bilirubin, providing the values most closely to the time of genetic diagnosis. Isolated increased serum GGT activity >1.5 times upper limit of normal was seen in 40 patients and isolated AP activity >1.5 times upper limit of normal in 4 patients, respectively, and in 4 patients, laboratory results were available only during treatment with UDCA. Severity of chronic liver disease was evaluated including clinical signs of cirrhosis, imaging, TE, and liver biopsy. Noninvasive assessment of liver fibrosis was performed by TE in 53 patients. Eight patients presented with LSM ≥10.7 kPa, indicating advanced fibrosis (≥F3), and of these, 3 patients showed LSM ≥16.9 kPa, compatible with cirrhosis. In 5 patients, cirrhosis was diagnosed clinically and in imaging studies, and in one of these patients liver biopsy confirmed cirrhosis. In 2 patients liver biopsy showed advanced fibrosis (F3). Thus, in total, 11 patients (12%) of the study cohort were considered having advanced fibrosis (≥F3), and of these, 5 patients (5%) presented with cirrhosis.

Of 82 patients with available data on UDCA therapy, 59 patients (72%) received UDCA. Information on the

Table 2. Genotype distribution and association of selected variants in the hepatobiliary transporter genes *ABCB4* and *ABCB11* in patients with idiopathic cholestasis and controls

Polymorphism	Cholestasis, cohort, <i>n</i> (%)	Control, cohort, <i>n</i> (%)	Tests for association*		
			OR	χ^2	<i>p</i> value
ABCB4					
c.504T>C (rs1202283)	<i>n</i> = 57	<i>n</i> = 252			
CC	21 (36.8)	76 (30.1)			
TC	26 (45.6)	135 (53.6)			ns
TT	10 (17.5)	41 (16.3)			
c.711A>T (rs2109505)	<i>n</i> = 94	<i>n</i> = 251			
TT	3 (3.2)	6 (2.4)			
AT	12 (12.8)	68 (27.1)	1.48	4.33	0.037
AA	79 (84.0)	177 (70.5)			
ABCB11					
p.A444V (rs2287622)	<i>n</i> = 93	<i>n</i> = 252			
CC	39 (41.9)	84 (33.3)			
CT	46 (49.5)	126 (50.0)	1.51	4.13	0.042
TT	8 (8.6)	42 (16.7)			
c.3084A>G (rs497692)	<i>n</i> = 93	<i>n</i> = 252			
GG	22 (23.6)	68 (27.0)			
AG	54 (58.1)	115 (45.6)			ns
AA	17 (18.3)	69 (27.4)			
OR, odds ratio. * Armitage's trend test.					

effect of UDCA treatment was available in 27 patients, and all patients except 1 showed improvement of AP and/or GGT activities.

Transporter Genotype Distributions

Genotype frequencies of the study cohort with unexplained cholestasis were compared to healthy blood donors as well as to database results from the Genome Aggregation Database of the European population (gnomAD; <http://gnomad.broadinstitute.org/>). Polymorphisms were successfully genotyped in at least 98.8% of patients tested, and there was no deviation of genotype distributions from the Hardy-Weinberg equilibrium in the control population for each of the SNPs tested (all $p > 0.05$). In the cholestasis cohort, the *ABCB4* rs2109505 (c.711A>T) genotype distribution deviated from Hardy-Weinberg equilibrium ($p = 0.033$), consistent with an association between the trait and genotypes. Table 2 summarizes the genotype distributions of the *ABCB4* rs1202283 (c.504T>C), rs2109505 (c.711A>T), *ABCB11* rs2287622 (p.A444V), and rs497692 (c.3084A>G) variants. Genotyping results were available in 57 patients of the cholestasis cohort for the *ABCB4* rs1202283 (c.504T>C) SNP, in all 94 patients for the *ABCB4* rs2109505 (c.711A>T) SNP, and 93 patients for the *ABCB11* rs2287622 (p.A444V) and *ABCB11*

rs497692 (c.3084A>G) variants, respectively. The genotype distribution of the *ABCB4* rs2109505 (c.711A>T) polymorphism was significantly ($p = 0.037$) more frequent in patients with unexplained cholestasis than in healthy blood donors (Table 2). Importantly, the more prevalent genotype (AA) represents the risk variant of this polymorphism. When comparing the results to an identical number of European individuals from the gnomAD database, the risk variant was also significantly ($p = 0.017$) more prevalent in the study cohort of patients with unexplained cholestasis.

Genotype frequencies of the risk variant of the common *ABCB11* p.A444V (c.1331T>C) polymorphism were significantly ($p = 0.042$) higher in the cohort of patients with unexplained cholestasis than in healthy blood donors (Table 2). There was no difference of genotype frequencies when the cholestasis cohort was compared to the frequencies of the European population in the gnomAD database. Genotype frequencies in the study and control cohorts for variants *ABCB4* rs1202283 (c.504T>C) and *ABCB11* rs497692 (c.3084A>G) are shown in Table 2. Statistical analysis revealed no significant differences when comparing the genotype frequencies of these 2 SNPs between the study cohort of patients with unexplained cholestasis and the control populations.

In 34 of the 94 patients (36.2%) with idiopathic cholestasis both risk variants, the *ABCB4* rs2109505 (c.711A>T) and the *ABCB11* p.A444V (c.1331T>C) polymorphisms were present, whereas only 61 of 252 (24.2%) individuals of the control cohort of healthy blood donors carried this combination ($p = 0.031$). Patients with idiopathic cholestasis harboring both risk variants exhibited significantly higher AP (median 160 U/L, range 80–1147 U/L; $p = 0.01$) and AST activities (median 44 U/L, range 25–556 U/L; $p = 0.029$) than cholestatic patients not carrying any of the 2 variants (AP median 121 U/L, range 37–662 U/L; AST 37 U/L, range 6–1,093 U/L). In patients harboring both risk variants, GGT levels (median 239 U/L, range 38–2,567 U/L), ALT activities (median 78 U/L, range 22–1,443 U/L), and bilirubin concentrations (median 0.6 mg/dL, range 0.2–11.3 mg/dL) did not differ as compared to the group without these variants (GGT median 136 U/L, range 9–1287 U/L; ALT median 58.5 U/L, range 14–1,339 U/L; bilirubin median 0.5 mg/dL, range 0.2–45.0 mg/dL). The presence of jaundice, pruritus, and advanced fibrosis did not differ between cholestatic patients harboring both risk and patients without variants. None of the patients with chronic cholestasis were carriers of the tested pathogenic variants *ABCB11* rs72549402 (p.D482G), rs11568372 (p.E297G), *ATP8B1* rs121909100 (p.I661T), rs34018205 (p.E429A), and rs146599962 (p.N45T), respectively.

Additionally, genotype distributions were also analyzed in the subgroup of 40 patients with idiopathic cholestasis and isolated GGT elevation. The genotype distribution of the *ABCB4* rs2109505 (c.711A>T) polymorphism was significantly ($p = 0.047$) more frequent in this subgroup of patients with unexplained cholestasis as compared with healthy blood donors (online suppl. Table 1; see www.karger.com/doi/10.1159/000518203 for all online suppl. material) and European individuals in the gnomAD Database ($p = 0.033$), respectively. There were no differences for genotype distributions of the *ABCB4* rs1202283 (c.504T>C), *ABCB11* p.A444V (c.1331T>C), and *ABCB11* rs497692 (c.3084A>G) polymorphisms in comparison with controls, respectively (online suppl. Table 1).

Discussion

In the present study, we show that the common *ABCB4* c.711A>T and the *ABCB11* p.A444V variants are more prevalent in a large cohort of adult patients with chronic unexplained cholestasis than in healthy controls. This

finding adds further information to the role of hepatobiliary transporter variants in cholestatic liver disease.

In children, high impact variants of the *ABCB4* gene, strongly reducing transport function, result in severely impaired phosphatidylcholine excretion into bile and finally progressive fibrosis and biliary cirrhosis [4, 6, 26–28]. This phenotype, designated PFIC3, usually manifests in the first years of life and frequently progresses to decompensated cirrhosis with portal hypertension, necessitating liver transplantation in about half of the patients already in childhood [10]. Adult patients with *ABCB4* disease may present with various clinical phenotypes, including ICP and LPAC syndrome. LPAC syndrome is characterized by the onset of biliary symptoms before the age of 40 years, intrahepatic sludge or micro-lithiasis, and the recurrence of biliary symptoms after cholecystectomy [21, 22]. Furthermore, chronic unexplained cholestasis in adults has been linked to *ABCB4* mutations in a pilot study of Ziol et al. [16]. About one-third of the 32 patients carried heterozygous *ABCB4* mutations, and these were associated to more severe fibrosis. *ABCB4* mutations were also found causative for chronic ductopenic cholestatic liver disease in adulthood in a family study from Romania [15] and in cholestatic patients from Italy [29]. In a study from Germany, a disease causing *ABCB4* mutation was detected in 20% of 215 cholestatic patients [14]. Previous studies showed that patients with *ABCB4* disease and onset in childhood with a PFIC3 phenotype often exhibit deleterious homozygous mutations, whereas in cases with adult onset, predominantly heterozygous mutations or less severe types with predicted residual *ABCB4* activity are present [10, 14, 16, 26, 27]. There is also experimental evidence that the disease course depends on the remaining function of the *ABCB4* transporter [30]. Accordingly, a classification of *ABCB4* mutations has been proposed based on the genetic variation, which may help tailoring future treatment [31–34].

Milder forms of chronic cholestatic liver disease in children are linked to heterozygous *ABCB4* variants, suggesting that moderate impairment of *ABCB4* function mediates susceptibility for liver disease in pediatric patients [35, 36]. Next to rare pathogenic gene variants, common polymorphisms contribute to manifestation of multiple diseases, either as nonsynonymous SNPs, which result in an amino acid exchange, or synonymous SNPs, which may affect mRNA processing and splicing and thereby protein levels and conformation [37]. Common *ATP8B1*, *ABCB11*, and *ABCB4* polymorphisms have been associated with cholestatic diseases such as ICP [17, 38].

ICP typically manifests in the second or third trimester of pregnancy and is characterized by pruritus, elevated serum ALT activities, and fasting bile acid concentrations, with an increase >100 µmol/L being correlated with worse fetal outcome [1, 39]. The synonymous *ABCB4* c.711A>T SNP has been shown to be more prevalent in a series of 184 women with ICP from the UK than in controls [18] and was also associated with the severe form of ICP in Swedish women [19]. In a subsequent large study in patients with ICP of European origin, the *ABCB4* c.711A>T SNP was the most significant of 6 polymorphisms, identified as key signal and confirmed in a second cohort [17]. The importance of *ABCB4* gene variants is underlined by the large-scale whole-genome sequencing study of the Icelandic population, which revealed an association of rare, deleterious variants with early onset of gallstone disease, ICP, cirrhosis, and hepatobiliary cancer. Moreover, the c.711A>T SNP was also associated with ALT, AST, and GGT serum activities in Iceland [20]. In our study, the genotype frequency of the *ABCB4* c.711A>T risk variant was higher in the cohort of patients with idiopathic cholestasis than that of study controls as well as the gnomAD database. Also in the subgroup of cholestatic patients with isolated GGT elevation, the *ABCB4* c.711A>T risk variant was more frequent than that in controls. Of note, the more prevalent common allele represents the risk allele for this SNP, a phenomenon which may appear in complex traits. The *ABCB4* c.711A>T SNP is not predicted to affect mRNA splicing [14, 17], hitherto its exact pathophysiological mechanisms remain to be elucidated.

ABCB4 disease covers a wide range of clinically phenotypes, ranging from severe forms of PFIC in children, chronic cholestasis in adults, LPAC syndrome, and ICP to GGT elevations without signs of liver fibrosis [6, 28]. The clinical picture correlates in general to the severity of genetic variation, with cirrhotic children carrying homozygous *ABCB4* high impact variants and less severe, mostly (compound) heterozygous variants, in adult forms of a PFIC-like phenotype [10, 14]. The GWAS study from the Icelandic population, which showed a correlation of the *ABCB4* c.711A>T SNP to elevated liver enzymes, suggests a general role of *ABCB4* variants and phosphatidylcholine deficiency in liver disease [20]. Polymorphisms, such as the *ABCB4* c.711A>T SNP, may represent susceptibility factors for liver disease, in particular for cholestatic disease courses. External factors and/or interaction with additional procholestatic gene variants may determine disease severity.

The *ABCB11* p.A444V variant has been associated with ICP and drug-induced liver injury [40–42]. In our

study, the p.A444V variant was more prevalent in the cohort of cholestatic patients than the control group; however, this difference was not seen in comparison to genotype distributions in the gnomAD database and also not in the subgroup of cholestatic patients with isolated GGT elevation. This common polymorphism may play a role in the absence of mutations with higher impact, similar to findings by Dröge et al. [14]. In the cholestatic group, 36% of the patients harbored the *ABCB4* c.711A>T and *ABCB11* p.A444V risk variants, which was significantly higher than those in controls. Cholestatic patients exhibiting both risk variants displayed higher AP and AST activities than those in cholestatic patients without these risk variants, which may possibly indicate a synergistic procholestatic effect of these variants; however, clinical parameters did not differ significantly between the 2 groups.

In our study, we were able to include a relatively large cohort of adults with unexplained cholestasis, a patient group rarely described in the literature. Pruritus and jaundice were the major clinical symptoms; however, half of the patients presented without clinical symptoms and were diagnosed based on abnormal laboratory parameters, which has also been seen in the study of Ziolk et al. [16]. A history of ICP was reported in 17% of women with former pregnancies, whereas in the general population ICP affects only 0.1–2% of pregnancies [39]. The majority of cholestatic patients received a therapy with UDCA and most of them showed an improvement of laboratory parameters. Given the beneficial side effect profile, UDCA appears to be a good choice for first-line medication in adult patients with unexplained chronic cholestasis. However, this was a retrospective analysis, and we cannot draw conclusions from our study concerning the effect of UDCA on progression of fibrosis. In children with the PFIC3, response to UDCA is often only intermediate and progression to cirrhosis is common [10, 27]. In adults, a 23-year-old patient with chronic cholestasis and 2 *ABCB4* missense mutations with residual transporter expression showed disappearance of advanced fibrosis after 9 years of UDCA therapy [43]. More specific therapies, taking into account the type of *ABCB4* mutation, are under investigation [33].

Limitations of the study are lack of data on the evolution of liver disease in this patient group during long-term follow-up and no systematic evaluation of liver biopsies. Further studies, including whole exome sequencing analysis of genes, may provide a more detailed picture of genetic variation in patients with unexplained chronic cholestasis, since variants in further genes such as *MYO5B*,

NR1H4, *TJP2*, and *ABCC12* have recently been identified in patients with cholestatic liver disease [11, 44].

In conclusion, we showed that the common *ABCB4* c.711A>T and the *ABCB11* p.A444V variants are more prevalent in adult patients with chronic unexplained cholestasis than in healthy controls. These variants may therefore confer susceptibility and contribute to the development of chronic cholestatic liver disease next to rare disease-causing mutations.

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Statement of Ethics

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of Ärztekammer des Saarlandes (271/11) and Leipzig (033/18-ek). All patients in the study provided written informed consent.

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Conflict of Interest Statement

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Author Contributions

C.J. contributed to study design, acquisition, analysis and interpretation of data, drafting of the manuscript, and final revision; C.J. contributed to acquisition, analysis and interpretation of data and critical revision of the manuscript for important intellectual content; J.F. contributed to acquisition and analysis of data and critical revision of the manuscript; T.B. contributed to study design and critical revision of the manuscript; F.L. contributed to study design, interpretation of data, and critical revision of the manuscript. Each author approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further enquiries can be directed to the corresponding author.

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