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Carriers of *ABCB4* gene variants show a mild clinical course, but impaired quality of life and limited risk for cholangiocarcinoma

Short title: *Clinical course in ABCB4 deficiency*

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Abbreviations:

ABCB4	adenosine triphosphate binding cassette subfamily B member 4
ADPKD	autosomal dominant polycystic kidney disease
CA	cholangitis activity
CCA	cholangiocarcinoma
CIC	oral contraceptive-induced cholestasis

ERCP	endoscopic retrograde cholangiopancreaticography
HA	hepatitis activity
HRQOL	Health-Related Quality of Life
ICP	intrahepatic cholestasis of pregnancy
IQR	interquartile range
LPAC	low-phospholipid-associated cholelithiasis
MDR 3	multidrug resistance 3
NOS	not otherwise specified
PBC	primary biliary cholangitis
PFIC3	progressive familial intrahepatic cholestasis type 3
PHSF	persistent hepatocellular secretory failure
PSC	primary sclerosing cholangitis
PTCD	percutaneous transhepatic biliary drainage
SD	standard deviation
SF-36	36-Item-Short-Form-Health-Survey
UDCA	ursodeoxycholic acid
US	ultrasound
VAS	visual analogue scale

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Abstract

Background and Aims: ABCB4 deficiency may lead to progressive familial intrahepatic cholestasis type 3 (PFIC3), biliary cirrhosis, low-phospholipid-associated cholelithiasis (LPAC), intrahepatic cholestasis of pregnancy (ICP), oral contraceptive-induced cholestasis (CIC) or may remain asymptomatic. The long-term course, quality of life and histology were investigated in *ABCB4* deficiency.

Methods: Adult carriers of *ABCB4* gene variants from two regional academic centers were analyzed by history taking, electronic patient files, physical examination, blood analysis, abdominal ultrasound (US) and liver elastography. Patients completed a 36-Item-Short-Form-Health-Survey (SF-36) for quality of life and a Visual Analogue Scale (VAS) for pruritus. Available liver specimens were re-classified according to the Nakanuma scoring system, so far validated for primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) only. Quality-of-life-data were compared to published data of patients with PBC, PSC and the general population.

Results: Sixty-seven patients were identified, 64 (96%) were alive at time of analysis, 62 (93%) were (at some time) treated with ursodeoxycholic acid. Two patients died of cholangio-carcinoma, one of decompensated biliary cirrhosis. Three additional deaths of cholangiocarcinoma were reported in first degree relatives. Transplant-free survival was 91% (median follow-up 14 years). Liver stiffness was normal (<6.3 kPa) in 75%, intrahepatic stones were detected at US in 33% and micro-calcifications in 22% of cases. Quality of life (n=48) was lower than in the general population particularly in energy/fatigue and general health domains and comparable to that in PSC. Staging according to Nakanuma in 15 specimens reflected the clinical course.

Conclusions: ABCB4 deficiency has a mild clinical course, but impaired quality of life and limited risk of cholangiocarcinoma. The Nakanuma scoring system appears feasible for histological evaluation in ABCB4 deficiency.

Lay summary

The long-term course and prognosis of adult carriers of ABCB4 variants/mutations, a liver and bile duct disorder called 'ABCB4 deficiency', with impaired secretion of protective phospholipids into bile is not well studied. We followed 51 women and 16 men with ABCB4 deficiency over an average of 14 years and found that the vast majority had a mild clinical course and no progressive liver and bile duct disease, but impaired quality of life. A minority developed decompensated cirrhosis, liver and bile duct cancer.

Keywords

ABCB4 deficiency; intrahepatic cholestasis of pregnancy (ICP); low phospholipid-associated cholelithiasis (LPAC); persistent hepatocellular secretory failure (PHSF); quality of life;

INTRODUCTION

The adenosine triphosphate binding cassette subfamily B member 4 (*ABCB4*) gene, located on chromosome 7q21, encodes the multidrug resistance 3 (MDR 3) protein.[1] MDR3 moves phosphatidylcholine from the inner to the outer leaflet of the canalicular hepatocyte membrane. Phosphatidylcholine forms mixed micelles with bile acids and cholesterol and, thereby, alleviates bile acid toxicity in the biliary tree.[1] *ABCB4* harbors numerous missense mutations, which may cause a broad spectrum of liver diseases.[2] Progressive familial intrahepatic cholestasis type 3 (PFIC3) is an autosomal recessive disease, usually presents in childhood and is caused by bi-allelic mutations in the *ABCB4* gene. Heterozygous mutations, leading to impaired ABCB4 function (ABCB4 deficiency), but no complete loss of function, have been associated with low-phospholipid-associated cholelithiasis (LPAC) syndrome, intrahepatic cholestasis of pregnancy (ICP) and oral contraceptive-induced cholestasis (CIC).[1,2] Some patients with a proven *ABCB4* gene variant do not fulfill the diagnostic criteria of the described phenotypes (in our cohort defined as ABCB4 deficiency not otherwise specified (NOS)). LPAC syndrome is a peculiar form of intrahepatic cholelithiasis occurring in young adults characterized by at least two of the following criteria: (1) age <40 years at onset of biliary symptoms; (2) intrahepatic echogenic foci or hepaticolithiasis; (3) recurrence of biliary symptoms after cholecystectomy.[3] The diagnosis of intrahepatic cholestasis of pregnancy (ICP) is based on (1) pruritus in pregnancy, (2) elevated serum alanine-aminotransferase activities and fasting bile acid levels, and (3) exclusion of other causes of liver dysfunction or itching and is confirmed when serum liver tests completely normalize after delivery.[4] CIC is a cholestatic drug-induced liver injury (DILI) and suspected when cholestasis develops after intake of oral contraceptives. Ursodeoxycholic acid (UDCA) is the only suggested medical therapy for patients with *ABCB4* deficiency. UDCA stimulates impaired hepatocellular secretion of hydrophobic bile acids and stabilizes the "biliary HCO_3^- umbrella".[5] Moreover, UDCA can prevent cholesterol gallstone formation and gallstones can decrease in size or disappear under UDCA treatment.[6] Clinical relevance of follow-up in adults with ABCB4 deficiency remains uncertain. However, clinical cases of cholangiocarcinoma in association with ABCB4 deficiency have been reported in literature.[7] The objectives of this study were to evaluate the long-term clinical course in patients with an *ABCB4* gene variant associated liver disease, their quality of life and the feasibility of a histological scoring system, validated in biliary diseases

(primary biliary cholangitis, PBC and primary sclerosing cholangitis, PSC) to systematically stage liver specimens.

METHODS

Study population

Adult patients with a proven *ABCB4* gene variant, who visited during the past two decades the outpatient liver clinic of two academic medical centers in the Netherlands (Amsterdam University Medical Centers, location AMC, and Erasmus Medical Center, Rotterdam) were included. Patients were identified in the electronic record systems of both hospitals. The study was approved by the Medical Research and Ethics Committee of both centers and all patients gave written informed consent.

Data collection

Clinical data

Clinical, biochemical and imaging findings (i.e. physical examination, laboratory and genomic DNA tests, abdominal ultrasound and liver stiffness measurement) were collected retrospectively from patients' records and censored at the date of the last visit. Patients were asked to complete two questionnaires in order to evaluate the Health-Related Quality of Life (HRQOL): 36-Item Short Form Health Survey (SF-36)[8], concerning quality of life and Visual Analogue Scale (VAS)[9], regarding pruritus. The SF-36 questionnaire includes eight domains: role limitations due to physical health or to emotional problems, pain, energy/fatigue, emotional wellbeing, social functioning, general health and perceived health change. Each SF-36 subscale score ranges from 0 to 100, a higher score indicates a better quality of life. Itch intensity was measured on a VAS with 0 indicating no itch and 10 indicating the worst itch possible. To compare quality of life to reference cohorts as general population and patients with other forms of chronic cholestatic liver diseases, previously published data were used (general Dutch population[10], PBC[11] and PSC[12]). Results of laboratory investigations, abdominal ultrasound (Philips EpiQ5), liver stiffness (FibroScan®) and questionnaires were collected.

Histological analysis

Original liver specimens of patients with a known *ABCB4* gene variant were collected from the archives of the pathology departments. Historical samples (obtained by needle biopsy, liver

resection or liver explant after transplantation) were blindly re-analyzed by two pathologists specialized in liver pathology (J.V., M.D.), who scored the liver specimens in tandem. Samples with a total number of ≥ 6 portal tracts were considered as reliable to assess. However, considering the descriptive character of this study, we accepted to include one liver biopsy with only 4 portal tracts. All samples were formalin-fixed and paraffin-embedded, and stained with hematoxylin–eosin and collagen stain (AZAN, Sirius Red, trichrome and/or Elastica von Giesson). Rhodanin, orcein and PAS-D for detection of copper binding protein were used, depending on which stain was available in the archives. Since sclerosing cholangitis of small bile ducts has been described in ABCB4 deficiency and no validated histopathological system for ABCB4 deficiency exists, we classified the samples according to the Nakanuma scoring system[13], which is validated for PBC[14] and PSC[15]. The Nakanuma scoring system includes degree of fibrosis (0-3), bile duct loss (0-3) and deposition of orcein-positive granules (0-3) for copper detection, together determining the final Nakanuma stage (1-4). Disease activity ('grading') is also evaluated with cholangitis activity [CA] (including duct-oriented lymphoplasmacytic infiltration and epithelioid granuloma) and chronic active hepatitis [HA], representing necro-inflammatory and immune-mediated lesions[13]. Calcifications (intraductal, parenchymal), cholesterol clefts and dysplasia were evaluated with respect to potential associations with cholangiocarcinoma[7]. "Onionskin-like fibrosis", formerly considered as pathognomonic histological feature of PSC, was assessed as well.

Statistical analysis

Patient characteristics were evaluated using descriptive statistics. Continuous data were expressed as median with interquartile range (IQR), quality of life data as mean with standard deviation (SD). Percentages were used for categorical variables. Wilcoxon signed-rank test was used to compare liver stiffness in individual patients. Mann-Whitney test and Kruskal-Wallis test were used to compare differences of stiffness between different phenotypes and ultrasound characteristics. Association between SF-36 domains and clinical characteristics was assessed by linear regression. Therefore, mean level of each SF-36 domain in the dataset was compared with each of PBC[11], PSC[12] and the general population[10] separately, using Student's t-test. All statistical

testing was performed two-sided at the 0.05 significance level using SPSS software v 25.0 (IBM, Armonk, NY, USA).

RESULTS

Patient characteristics

A total of 67 patients were evaluated, 16 (24%) were male and 51 (76%) were female (Table 1). Median age at time of evaluation was 43 years (IQR 34-51), 64 patients (96%) were alive at time of analysis. Mean time between first presentation and time of our cohort analysis was 14 years (IQR 7-19). The majority of evaluated patients were first diagnosed with LPAC (n=43, 64%), 11 patients (16%) with ICP, 4 patients (6%) with PFIC3, 2 patients (3%) with CIC and, notably, 1 patient (2%) with persistent hepatocellular secretory failure (PHSF). In addition, 6 patients had a proven *ABCB4* gene variant, without fulfilling the diagnostic criteria of the described phenotypes (ABCB4 deficiency NOS). Some patients developed multiple phenotypic characteristics associated with ABCB4 deficiency during the course of their disease (Table 1). The median age at diagnosis, established as the date of proven *ABCB4* gene variant, was 37 years (IQR 28-46). The first clinical presentation was for 63% caused by symptomatic gallstone disease, for 18% by ICP or CIC, for 12% by biochemical cholestasis of unknown cause and for 7% as consequence of family screening. The median period between first presentation and definite diagnosis of ABCB4 deficiency was 7 years (IQR 1-15). Thirty-seven patients underwent cholecystectomy at a median age of 29 years (IQR 12–35). Notably, 73% suffered from cholangitis after cholecystectomy of whom 74% required additional treatment (endoscopic retrograde cholangiopancreaticography (ERCP), percutaneous transhepatic biliary drainage (PTCD)). A positive family history concerning hepatobiliary diseases in first degree relatives (elevated liver tests, symptomatic gallstones, pruritus during pregnancy, cirrhosis, cholangiocarcinoma) was reported by 49% of patients and 28% had a first degree relative carrying a proven *ABCB4* gene variant. Strikingly, three patients had a first degree family member with a history of cholangiocarcinoma (CCA), of whom one with a proven *ABCB4* gene variant. Serum liver tests were normal in 66% of patients. When elevated, gamma-glutamyl transferase was particularly increased (median 67 U/l, IQR 29–140), known for *ABCB4* deficiency-related liver disease, in contrast to other forms of PFIC.[1,2]

Patients underwent abdominal ultrasound that showed hepaticolithiasis in 33%, intrahepatic microcalcifications in 21%, gallbladder stones in 10%, signs of portal hypertension and hepatomegaly in 3%, each, and no abnormalities in 38%. When using a high-resolution probe

(L4), intrahepatic microcalcifications appeared as longitudinal hyperechoic stripes within the bile duct wall (Figure 1A,B). Intrahepatic calcifications were diagnosed as hepaticolithiasis when a dorsal shadow on ultrasound was clearly seen (Figure 1C,1D). Remarkably, intrahepatic microcalcifications were not only detected in LPAC, but also in 4 patients with ICP, 1 patient with PHSF and 2 patients with ABCB4 NOS. These patients were asymptomatic, without liver test abnormalities and with normal median liver stiffness (6.0 kPa, IQR 4.0-8.2). MR imaging of the liver and bile ducts was performed in 25 patients (37%), showing hepaticolithiasis in 32% of cases, gallbladder stones in 12%, intrahepatic bile duct dilatation in 56%, signs of portal hypertension and hepatomegaly in 4%, each, and no abnormalities in 44%. A liver stiffness measurement was performed in 90% of all patients with a median liver stiffness of 6.8 kPa (IQR 4.0-7.6). Only liver stiffness measurements obtained with at least ten successful measurements, with a success rate above 60% and an IQR below 33% were considered reliable and were used for analysis. No association was observed between liver stiffness and different phenotypes (p=0.73). In addition, no differences were observed between various ultrasound features (no abnormalities versus intrahepatic stones/microcalcifications) and liver stiffness (p=0.14). In patients who underwent several liver stiffness measurements over years (n=29), liver stiffness did not increase (p=0.34); the median interval between these measurements was 3 years (IQR 2–4).

Almost 80% of patients were under UDCA treatment with a median dosage of 13 mg/kg (IQR 12-15) and median duration of 8.5 years; 13% of patients had been treated with UDCA in the past and stopped treatment because of normalization of liver tests or absence of symptoms and only 7% of patients were never treated with UDCA probably because of no laboratory / imaging abnormalities (2 patients with LPAC, 1 with ICP, 1 with CIC and 1 with ABCB4 NOS). Among ICP patients, 95% were treated with UDCA. Of the 67 analyzed patients, three patients underwent liver transplantation; one patient with LPAC syndrome because of decompensated liver cirrhosis (760G>A, 1546A>G, 2363G>A; Ala254Thr, Met516Val, Arg788Gln), with hepatocellular carcinoma in the explanted liver. One patient suffered from decompensated liver cirrhosis due to PFIC3 (c.100del, c.2800G>A; p.Thr34Argfs*4 and p.Ala934Thr). One patient underwent a combined liver and kidney transplantation as a result of congenital liver fibrosis in the context of an autosomal dominant polycystic kidney disease (ADPKD, genotype not determined), in combination with LPAC syndrome (1769G>A; p.Arg590Gln). This patient has no family history of

ABCB4 deficiency. Three patients died in our cohort over a period of 20 years. Two patients died as a consequence of cholangiocarcinoma: one patient at the age of 71 years with intrahepatic cholangiocarcinoma and one patient at the age of 47 years with perihilar cholangiocarcinoma. Both were diagnosed with LPAC syndrome (c1405A>T; p.Arg469Trp; c.1268A>C; p.Gln423Pro) and were treated with UDCA at a daily dose of 13mg/kg. One of these patients had a son diagnosed with LPAC syndrome, the other patient has 2 sisters who underwent a cholecystectomy at < 30 years of age. One patient with PFIC3 (c.523A>G and c.1769G>A; p.Thr175Ala and p.Arg590Gln) died due to complications of decompensated cirrhosis on the waiting list for liver transplantation.

The majority of patients (52.2%) has a known pathogenic (class 5) mutation. An overview of the class of mutation and diagnosis is presented in Table 2. Remarkably, all PFIC3 patients had a class 5 mutation and almost all patients with ABCB4 NOS as well, in addition the majority of ICP patients were also diagnosed with a class 5 mutation. However, no correlation between the class of mutation and severity of disease (Fibroscan^{R} ($p = 0.14$), quality of life domains except for social functioning domain ($p = 0.04$)) has been observed. We identified 9 new gene variants which were not described previously. Two variants were categorized as class 5 mutations (pathogenic) and two as class 4 mutations (likely pathogenic) (Table 3). A detailed Table S1 describing the genotype of each patient is attached in the supplementary appendix.

Quality of life

The SF-36 and pruritus VAS questionnaires were completed by 48 patients and were compared to the reference groups (patients with PBC and PSC and the general population; Table 3). The majority of the SF-36 domains (pain, physical functioning, role limitations due to physical health or emotional problems, emotional wellbeing) showed no impairment of health-related quality of life (HRQoL) in patients with ABCB4 deficiency when compared to the general population (Table 3). However, patients reported a lower quality of life in the energy/fatigue and general health domains ($p<0.001$; Table 3). Remarkably, no differences in HRQoL were observed between patients with ABCB4 deficiency and patients with PSC, except for the pain domain ($p=0.009$; Table 3). In contrast, patients with PBC reported a lower quality of life in all domains, except for

the energy/fatigue domain, which was comparable in the three patient groups. The domain of emotional wellbeing was equal in patients and the general population. None of the SF-36 domains showed a correlation with gender, age, phenotype of disease, ultrasound characteristics, current UDCA treatment, liver stiffness or cholecystectomy. In contrast to PBC and PSC, pruritus did not seriously affect the vast majority of patients with ABCB4 deficiency since 50% of the study population had a pruritus VAS score of 0, a VAS score ≤ 5 was scored by 44% and only 6% experienced pruritus with an intensity greater than 5 (Table 3). Of these 5 patients, 4 patients were diagnosed with LPAC syndrome and 1 with ICP. Two of the patients had a class 3 mutation, 2 patients a class 5 mutation and 1 patient a class 4 mutation.

Histopathological analysis

In total 25 patients had undergone a liver biopsy for various reasons in the past (i.e. suspicion of small duct PSC, cholestasis during pregnancy, serum liver test abnormalities). Fifteen of the biopsies were available and suitable for evaluation according to the Nakanuma scoring system (Supplementary Table 2S). These samples contained more than 6 portal tracts except for 1 biopsy with 4 portal tracts. Ten biopsies could not be evaluated because of low tissue quality or limited size (< 4 portal tracts in 5 patients)[13]. The median size of the samples was 22.8mm (range 10-35mm). The median duration from first presentation to the time of liver biopsy was 3 years (IQR 1-16 years); 8 specimens were from LPAC patients, 3 from patients with PFIC3, 2 from patients with ICP and 1 from a patient with CIC. According to the Nakanuma scoring system, 53% of the patients had a fibrosis score of 0 and 27% had a score of 1, thus 80% did not show advanced fibrosis (compatible with elastography, see above). Copper staining, a prognostic marker in PBC, was absent in the majority of patients (67%). Four patients had a bile duct loss score of 1, 4 a score of 2, but the others (47%) did not show any bile duct loss. Overall 80% of the patients were scored as Nakanuma stage 1 or 2 (no or mild disease), one patient as stage 3 and two patients as stage 4. No association between Nakanuma stage and class of mutation was observed (p=0.74). Concerning the activity items, 93% of the samples showed a cholangitis activity grade 1 or 2, whereas 80% did not show any hepatitis activity (interface and/or lobular). Calcifications,

cholesterol cleft and dysplasia were not detected in the analyzed specimens. Onion skin fibrosis was detected in 2 resection specimens (Figure 2) as reported previously[16].

DISCUSSION

This cohort study, with a median follow up of 14 years, shows that ABCB4 deficiency based on heterozygous gene variants in general has a mild clinical course. The majority of patients (75%) had no signs of liver fibrosis with a median liver stiffness <6.8 kPa and no progression of fibrosis during follow up. In line with previous reports we did not find a correlation between genotype and phenotype. However, this study also shows that ABCB4 gene variants can cause serious illness with important consequences such as decompensated biliary cirrhosis with or without hepatocellular carcinoma and cholangiocarcinoma as observed in our patient cohort and previously described in literature. Moreover, in our cohort 3 patients had to undergo a liver transplantation.

An elevated risk of cholangiocarcinoma in association with ABCB4 deficiency has been assumed previously[7], still an association of specific ABCB4 gene variants with development of cholangiocarcinoma is unlikely. The ABCB4 gene variants of our patients with cholangiocarcinoma differed from those published previously. Remarkably, in 3 patients of our cohort a positive family history for cholangiocarcinoma in first degree family members was reported. Apparently, the current state of knowledge does not allow to predict the clinical course of ABCB4 deficiency and the risk of progressive fibrosis or cholangiocarcinoma[1,2,7,17]. Therefore, an evidence-based advice for screening for cholangiocarcinoma is currently not possible.

We previously defined the rare and severely invalidating 'persistent hepatocellular secretory failure' (PHSF) with intense jaundice and itch after transient administration of certain medications or transient occlusion of bile ducts by stones or tumors.[18] We had identified defective gene variants/mutations in the canalicular transporter genes *ATP8B1* or *ABCB11* of 3 patients with PHSF as potential genetic risk factor when 6 of 13 patients with PHSF were tested.[18] In the present cohort, one patient with ABCB4 deficiency was diagnosed with PHSF, to the best of our knowledge the first patient with an *ABCB4* variant and PHSF described. This observation suggests a potential role for ABCB4 as for other defective canalicular transporters like ATP8B1 and ABCB11 in the development of PHSF.[18]

Although *ABCB4* deficiency is generally considered as a mild disorder, the present study provides important novel insight in the quality of life of patients with *ABCB4* deficiency. Patients reported

lower quality of life in some domains compared to the general population. In particular domains, i.e. energy / fatigue, social functioning and perception of general health, data were comparable to patients with PSC. In striking contrast, however, to PSC and PBC, pruritus does not play a (major) role in ABCB4 deficiency, a remarkable finding of this study.

Concerning liver histology, ABCB4 deficiency can lead to a form of sclerosing cholangitis as previously reported,[16] mainly involving small bile ducts and thereby mimicking the ill-defined 'small duct PSC' without cholangiographic abnormalities. Concentric periductal fibrosis ("onion skin fibrosis") was detected in 2 of our resection specimens contributing to the concept that onion skin fibrosis is a sign of various forms of sclerosing cholangitis and not pathognomonic for PSC. Hepatitis activity was not part of the histological picture in most patients.

The Nakanuma histological scoring system seems to be suitable to describe characteristics of ABCB4 deficiency since all the Nakanuma features were detected in liver specimens of our patients. Furthermore, 80% of the patients who were scored as stage 1 or 2 according to Nakanuma, had mild liver disease with liver stiffness <8 kPa and the only two patients who were scored as stage 4 underwent liver transplantation. Hence, the Nakanuma scoring system seems to reflect the clinical course of disease, though its prognostic value needs further evaluation. Regarding the potential association of CCA and ABCB4 deficiency, we do advise to evaluate dysplasia in liver specimens of patients with *ABCB4* deficiency, although this is not supported by the present data possibly due to the small sample size. One could assume that chronic inflammation in the biliary tree, as in PSC, might lead to a dysplasia-carcinoma sequence.[19] Calcifications and cholesterol clefts were also not seen in our biopsy specimens but were described in previous studies of liver specimens in ABCB4 deficiency.[7]

An interesting sonographic feature were intrahepatic microcalcifications (Figure 1), detected in a considerable number of asymptomatic patients with ABCB4 deficiency independent of the phenotype. By using high-resolution probes, these calcifications seem to be located in the bile duct wall, suggestive of cholesterol and calcium depositions. Larger calcifications clearly showed dorsal shadowing compatible with hepaticolithiasis. Predisposition for cholesterol gallstones in ICP and LPAC syndrome is a known phenomenon[1,20] and intrahepatic cholesterol stones often disclose *ABCB4* mutations due to impaired phospholipid release into bile[20], but these specific

intrahepatic microcalcifications have not been described in literature previously. It is unclear whether microcalcifications contribute to progression of ABCB4 deficiency.

The previously reported predominance of symptomatic ABCB4 deficiency in females was confirmed in our cohort (76%) although ICP formed only a minority of our patients. Estrogens and progesterone metabolites are known to affect ABCB4 expression and function[1] and might contribute to the clinical manifestation of ABCB4 deficiency.

Our cohort study has limitations. As ABCB4 deficiency is rare, the number of patients is limited. The heterogeneity of clinical manifestations and severity of disease impedes an evidence-based statement regarding treatment and follow-up of these patients.

In conclusion, patients with ABCB4 deficiency may present with a variety of clinical manifestations including LPAC syndrome, ICP, PHSF, biliary cirrhosis, hepatocellular and cholangiocarcinoma, but in a vast majority show a mild clinical course without progression over time. Notably, quality of life was diminished in social functioning, general health and energy/fatigue in comparison to the general population and comparable to patients with PSC. Concerning histology, the Nakanuma scoring system, meanwhile established in PBC and PSC, appears appropriate to systematically describe characteristic features in liver specimens of patients with ABCB4 deficiency. Prospective multicenter studies are needed to further explore the variability in clinical manifestations, the impact of diminished quality of life, the potential risk of cholangiocarcinoma and a potential alleviation of these burdens by medical intervention in ABCB4 deficiency.

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Tables

	n (% of total cohort)
Gender, male / female	16 (24%) / 51 (76%)
First disease manifestation Low Phospholipid-Associated Cholelithiasis (LPAC) * Intrahepatic Cholestasis of Pregnancy (ICP) ** Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3) Oral Contraceptive Induced Cholestasis (CIC) Persistent Hepatocellular Secretory Failure (PHSF) Not otherwise specified (NOS)	 43 (64%) 11 (16%) 4 (6%) 2 (3%) 1 (2%) 6 (9%)
Ursodeoxycholic acid treatment, current / past / never	53 (79.1%) / 9 (13.4%) / 5 (7.5%)
Cholecystectomy	37 (55.2%)
Cholangitis after cholecystectomy	27 (72.9%)
	Median (IQR)
Age at diagnosis, years (n= 67)	37 (28-46)
Age at cohort evaluation, years (n= 64)	43 (34-51)
Total bilirubin, µmol/L (n=67)	8 (7-10)
Gamma glutamyltransferase, U/L (n=67)	67 (22-140)
Alkaline phosphatase, U/L (n=67)	82 (68-95)
Aspartate aminotransferase, U/L (n=67)	27 (23-31)
Alanine aminotransferase,U/L (n=67)	44 (38-47)

FibroScan ®, kPa (n=60)	6.8 (4.0-7.6)

Table 1. Clinical and biochemical characteristics of 67 patients with ABCB4 deficiency

**LPAC: only LPAC: 31 (46.2%); LPAC and CIC: 3 (4.5%); LPAC and ICP: 5 (7.5%); LPAC, ICP and CIC: 2 (2.9%); LPAC, ICP and PHSF: 1 (1.5%); LPAC and PHSF: 1 (1.5%).*

*** ICP: ICP: 5 (7.4%); ICP and CIC: 6 (8.9%).*

Class of mutation							
	TOTAL	LPAC	ICP	PFIC3	CIC	ABCB4 NOS	PHSF
Number of patients (frequency %)							
1	6 (9%)	4 (9.3%)	1 (9.1%)	0	1 (50%)	0	0
2	2 (3%)	2 (4.7%)	0	0	0 (0%)	0	0
3	13 (19.4%)	10 (23.3%)	1 (9.1%)	0	1 (50%)	1 (16.7%)	0
4	8 (11.9%)	7 (16.3%)	1 (9.1%)	0	0	0	0
5	35 (52.2%)	19 (44.2%)	7 (63.6%)	4 (100%)	0	5 (83.3%)	0
Unknown	3 (4.5%)	1 (2.3%)	1 (9.1%)	0	0	0	1 (100%)

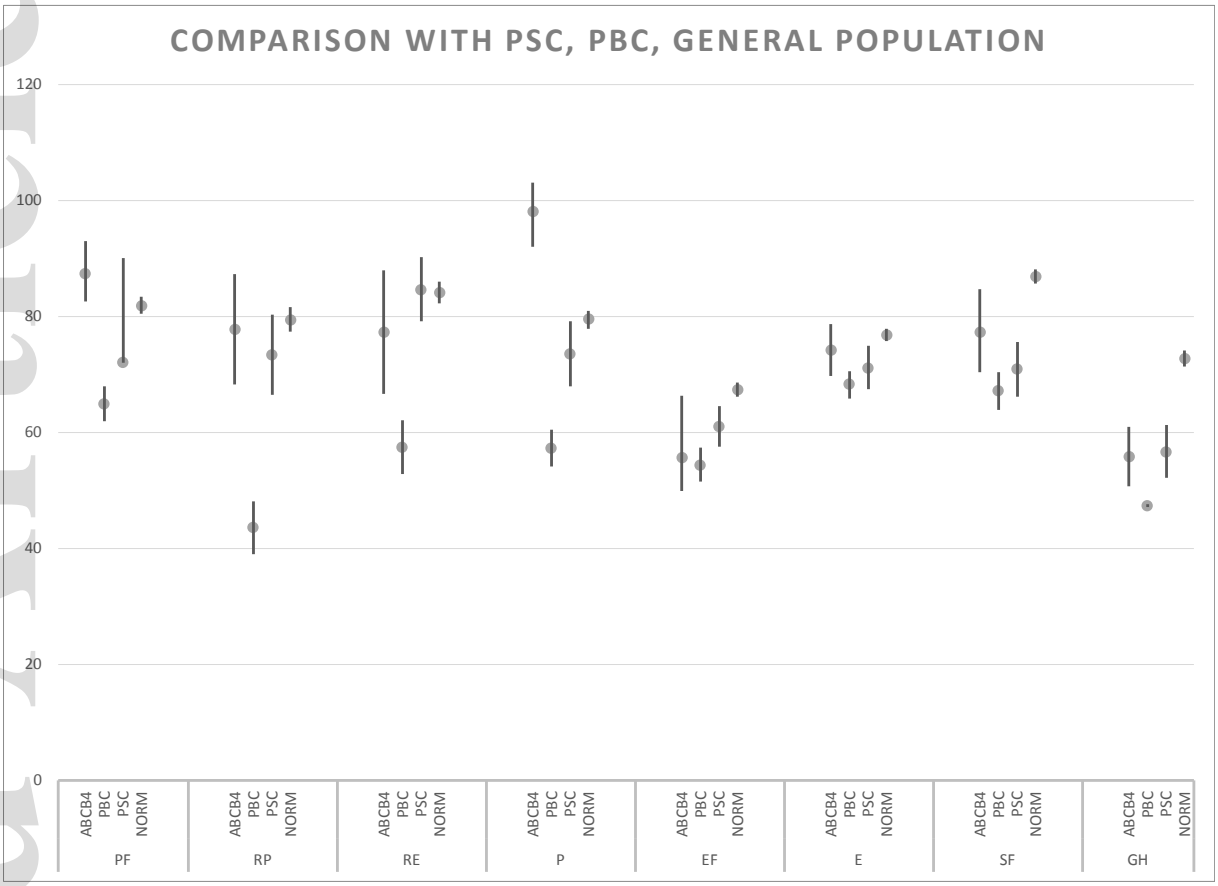
Table 2. Class of mutation of 67 patients with ABCB4 deficiency.

Classification of mutation: Class 1 = not pathogenic, Class 2 = likely not pathogenic, Class 3 = uncertain significance, Class 4 = likely pathogenic, Class 5 = pathogenic. LPAC = Low Phospholipid-Associated Cholelithiasis, ICP = Intrahepatic Cholestasis of Pregnancy, PFIC3 = Progressive Familial Intrahepatic Cholestasis type 3, CIC = Oral Contraceptive Induced Cholestasis, NOS = Not otherwise specified, PHSF = Persistent Hepatocellular Secretory Failure

Location and nucleotide change	Predicted effect amino acid	Class of mutation	Phenotype
3670C>T	p.(Arg122Cys)	4	LPAC, PHSF
1136G>A	p.(Ser379Asn)	3	LPAC
3214G>T	p.(Gly1072Cys)	4	ICP, CIC
c.1268A>C	p.(Gln423Pro)	3	LPAC
c.3597T>G	p.(Asp1199Glu)	3	LPAC
c.1546A>G	p.(Met516Val)	3	LPAC, ICP
c 135+5G>T	None	3	LPAC, ICP
c.2261del	p.(Phe754Serfs*3)	5	NOS
c.100del	p.(Thr34Argfs*4)	5	PFIC3

Table 3. New ABCB4 gene variants identified in the cohort of 67 patients with ABCB4 deficiency.

LPAC, Low Phospholipid-Associated Cholelithiasis; PHSF, Persistent Hepatocellular Secretory Failure; ICP, Intrahepatic Cholestasis of Pregnancy; CIC, Oral Contraceptive Induced Cholestasis; NOS, Not otherwise specified; PFIC 3, Progressive Familial Intrahepatic Cholestasis type 3. Classification of mutation: Class 1 = not pathogenic, Class 2 = likely not pathogenic, Class 3 = uncertain significance, Class 4 = likely pathogenic, Class 5 = pathogenic.



	PF	RP	RE	P	EF	E	SF	GH
PBC	**<0.001**	**<0.001**	**0.006**	**<0.001**	0.78	0.11	0.05	**<0.001**
PSC	0.85	0.39	0.12	**0.009**	0.09	0.44	0.21	0.81
NORM	0.09	0.89	0.09	0.09	**<0.001**	0.24	**0.001**	**<0.001**

Table 4. 36-Item Short Form Health (SF-36) scores in patients with ABCB4 deficiency in comparison to patients with PBC and PSC and the general population. Data are shown as mean with confidence interval. *PBC = primary biliary cholangitis, PSC = primary sclerosing cholangitis, NORM = general population, PF = physical functioning, RP = role limitations due to physical health, RE = role limitations due to emotional*

problems, P = pain, EF = energy/fatigue, E = emotional wellbeing, SF = social functioning, GH = general health, HC = health change

Figure legends

Figure 1. Abdominal ultrasound. **A**) Intrahepatic microcalcification. **B**) Intrahepatic microcalcification with high resolution probe view. **C, D**) Hepaticolithiasis. In contrast to the calcifications in Figure 1A and detail in 1B, the calcifications in Figure 1C and D clearly show a dorsal shadowing on ultrasound.

Figure 2. Periductal onion-skin fibrosis in a liver specimen of a patient carrying an ABCB4 gene variant, H&E (x200).

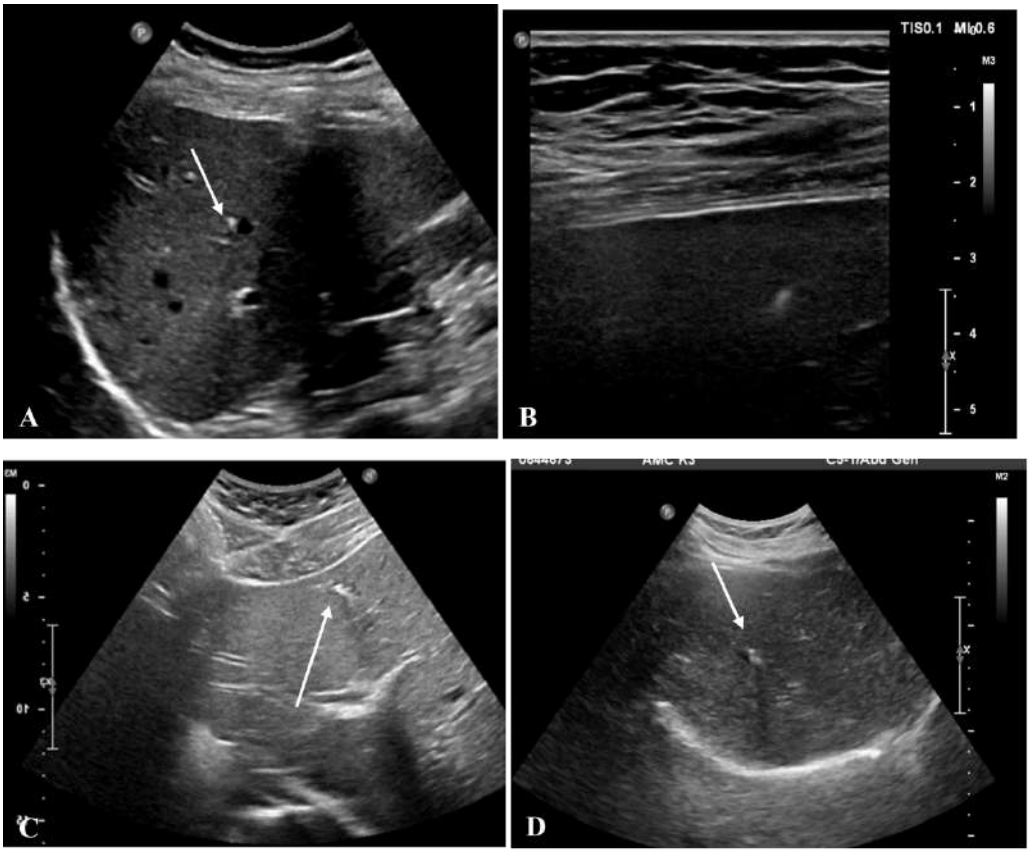


Figure 1. Abdominal ultrasound. **A**) Intrahepatic microcalcification. **B**) Intrahepatic microcalcification with high resolution probe view. **C and D**) Hepaticolithiasis. In contrast to the calcifications in Figure 1A and detail in 1B, the calcifications in Figure 1C and D clearly show a dorsal shadowing on ultrasound.

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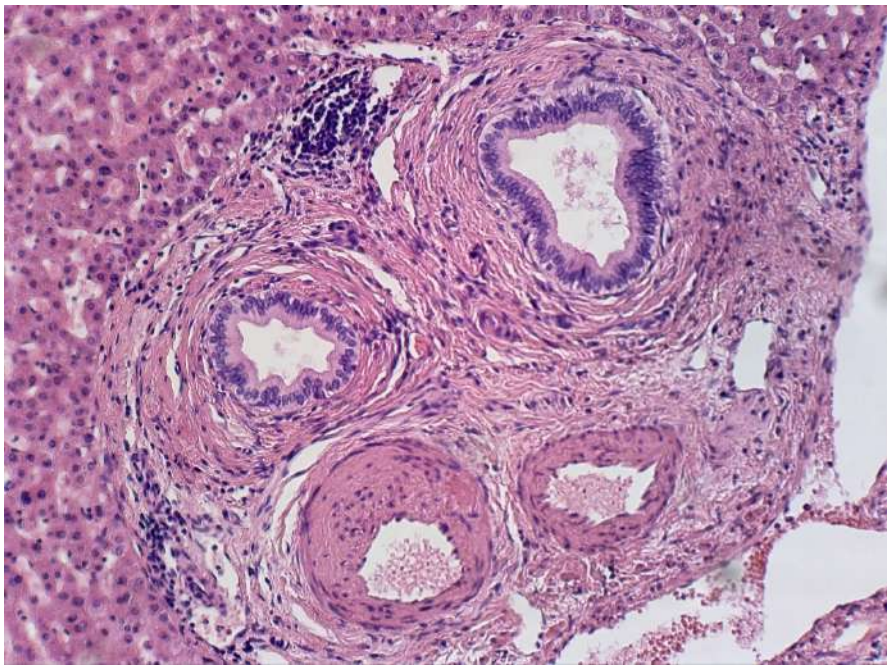

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