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ARTICLE

First description of *ABCB4* gene deletions in familial low phospholipid-associated cholelithiasis and oral contraceptives-induced cholestasis

Eric Pasmant*[,1,2], Philippe Goussard[1], Laetitia Baranes[1], Ingrid Laurendeau[2], Samuel Quentin[3], Philippe Ponsot[4], Yann Consigny[5], Olivier Farges[6], Bertrand Condat[7], Dominique Vidaud[1,2], Michel Vidaud[1,2], Jian-Min Chen[8] and Béatrice Parfait[1,2]

The wide clinical spectrum of the *ABCB4* gene (ATP-binding cassette subfamily B member 4) deficiency syndromes in humans includes low phospholipid-associated cholelithiasis (LPAC), intrahepatic cholestasis of pregnancy (ICP), oral contraceptives-induced cholestasis (CIC), and progressive familial intrahepatic cholestasis type 3 (PFIC3). No *ABCB4* mutations are found in a significant proportion of patients with these syndromes. In the present study, 102 unrelated adult patients with LPAC (43 patients) or CIC/ICP (59 patients) were screened for *ABCB4* mutations using DNA sequencing. Heterozygous *ABCB4* point or short insertion/deletion mutations were found in 37% (16/43) of the LPAC patients and in 27% (16/59) of the ICP/CIC patients. High-resolution gene dosage methodologies were used in the 70 negative patients. Here, we describe for the first time *ABCB4* partial or complete heterozygous deletions in 7% (3/43) of the LPAC patients, and in 2% (1/59) of the ICP/CIC patients. Our observations urge to systematically test patients with LPAC, ICP/CIC, and also children with PFIC3 for the presence of *ABCB4* deletions using molecular tools allowing detection of gross rearrangements. In clinical practice, a comprehensive *ABCB4* alteration-screening algorithm will permit the use of *ABCB4* genotyping to confirm the diagnosis of LPAC or ICP/CIC, and allow familial testing. An early diagnosis of these biliary diseases may be beneficial because of the preventive effect of ursodeoxycholic acid on biliary complications. Further comparative studies of patients with well-characterized genotypes (including deletions) and phenotypes will help determine whether *ABCB4* mutation types influence clinical outcomes.
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INTRODUCTION

ABCB4 (ATP-binding cassette subfamily B member 4) membrane transporter translocates phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane of the hepatocyte. This floppase activity makes phosphatidylcholine available for extraction into the canalicular lumen by bile salts. The *ABCB4* gene (MIM 171060) has a crucial role as evidenced by cholestatic liver diseases caused by its deficiency.[1] *ABCB4* is also known as multidrug resistance 3 gene (*MDR3*), a member of the MDR/TAP subfamily involved in multidrug resistance as well as antigen presentation. The human *ABCB4* gene is located on chromosome 7q21.1, contains 27 coding exons, and spans approximately 74 kb.[2] The pathophysiology of the *ABCB4* alterations resides in the lack of phospholipid protection in the bile against the detergent effect of the bile salts, resulting in damage to the biliary epithelium, bile ductular proliferation, and potential progressive portal fibrosis. As biliary cholesterol solubilization depends not only on the concentration of the sterol itself but also on the bile salt and phospholipid concentration, a decreased rate of phospholipid excretion can also be a cause of gallstone formation.

The wide clinical spectrum of the *ABCB4* deficiency syndromes in humans covers cholestatic disorders presenting from the neonatal period of life to late adulthood.[3–5] At least three distinct syndromes with variable severity have been clearly identified: progressive familial intrahepatic cholestasis type 3 (PFIC3; MIM 602347), low phospholipid-associated cholelithiasis (LPAC alias gallbladder disease 1, GBD1; MIM 60080), and familial intrahepatic cholestasis of pregnancy (ICP; MIM 147480). Evidences of *ABCB4* mutations have also been found in transient neonatal cholestasis,[6] or adult idiopathic biliary fibrosis or cirrhosis.[4,7–11]

A recessive inheritance pattern of PFIC3 has been observed.[7,12] Most *ABCB4* mutations in the patients with PFIC3 have been reported to be homozygous or compound heterozygous.[3,7,9,12–14] These mutations include missense and non-sense mutations, and short frameshift deletions or insertions. *ABCB4* mutations are associated with an absence or a weak level of the canalicular ABCB4 protein, and with a low level of biliary phospholipids.[7,12,15] Patients with PFIC3 usually

[1]Service de Biochimie et de Génétique Moléculaire, Hôpital Beaujon, Clichy, France; [2]UMR745 INSERM, Université Paris Descartes, Sorbonne Paris Cité, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France; [3]Plateforme Génomique, Institut Universitaire d'Hématologie, Université Paris Diderot and Laboratoire d'Hématologie APHP, Hôpital Saint-Louis, Paris, France; [4]Service de Gastroentérologie, Hôpital Beaujon, Clichy, France; [5]Cabinet Médical, Paris, France; [6]Service de Chirurgie digestive, Hôpital Beaujon, Clichy, France; [7]Service d'Hépato-Gastroentérologie, Hôpital Saint-Camille, Bry-sur-Marne, France; [8]INSERM U613 and EFS-Bretagne, Brest, France
*Correspondence: Dr E Pasmant, UMR745 INSERM, Université Paris Descartes, Sorbonne Paris Cité, Faculté des Sciences Pharmaceutiques et Biologiques, 4 avenue de l'Observatoire 75006, Paris, France. Tel: +33 1 53 73 97 25; Fax: +33 1 44 07 17 54; E-mail: eric.pasmant@gmail.com
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present at a few years of age and suffer from severe chronic and progressive cholestasis. Liver histology often reveals fibrosis with portal inflammation and strong bile duct proliferation in an early stage.[16] A characteristic high-serum gamma-glutamyltransferase activity is found in PFIC3. As a consequence of the cirrhosis, the PFIC3 patients are prone to gastrointestinal bleeding. About 50% of the patients need a liver transplantation. Interestingly, the other half may benefit from treatment with ursodeoxycholic acid (UDCA).[7]

Mutations in the *ABCB4* gene that may reduce but not eliminate or drastically decrease the protein (leaving residual activity of the transporter), have been shown to cause a variety of milder cholestatic phenotypes, including LPAC and ICP. LPAC is a peculiar form of biliary gallstone disease characterized by intrahepatic sludge and/or symptomatic cholesterol cholelithiasis in young adults (usually before 40 years). LPAC is typically associated with mild chronic cholestasis, recurrence of symptoms after cholecystectomy, and prevention of recurrence by UDCA. About one third to half of patients have missense, frameshift, or non-sense mutations – mostly heterozygous – in the *ABCB4* gene.[17–19] One of the hallmarks of LPAC is the response and remission induced by the UDCA therapy. Heterozygous *ABCB4* mutations were also identified in up to 15% of women suffering from ICP.[12,20,21–31] ICP is a reversible form of cholestasis that may develop in the third trimester of pregnancy, usually rapidly resolves after delivery and recurs in 45–70% of subsequent pregnancies.[27] The main symptoms are pruritus and, to a lesser extent, jaundice. Serum bile salt and aminotransferases levels are elevated. Increased incidence of fetal complications (including placental insufficiency, premature labor, and sudden fetal death) was reported in association with ICP. UDCA is the treatment of choice for ICP and produces relief from pruritus with improvement in liver tests, with no adverse maternal or fetal effects.[27,32,33] It is generally accepted that women who have suffered from ICP are also susceptible to the development of cholestasis on the use of oral contraceptives (oral contraceptives-induced cholestasis (CIC)).[34,35] Evidences of *ABCB4* heterozygous mutations have also been found in patients with drug-induced cholestasis or liver injury.[17,18,35,36]

Loss of function *ABCB4* mutations can present a large spectrum of cholestasis phenotypes, and genetic analysis of *ABCB4* can now be performed to confirm the diagnosis.[37] However, no *ABCB4* mutations are found in a significant proportion of patients. The lack of *ABCB4* mutation detection in remaining cases might be attributed to phenotyping errors, genetic heterogeneity, and inadequacy of genetic screening methods. In the present study, we have tested the last hypothesis by using recent high-resolution gene dosage methodologies in a large series of 102 adult patients with symptomatic intrahepatic cholelithiasis/cholestasis. Here, we describe for the first time *ABCB4* partial or complete deletions in patients with LPAC and CIC.

SUBJECTS AND METHODS

Patients

A panel of 102 clinically diagnosed index cases was phenotypically selected, by routine genetic diagnosis. All patients were examined by reference hepatologists or gastroenterologists. The full clinical and radiological available information were recorded. The written informed consent from each patient included in the study was obtained. In total, 59 unrelated adult women with ICP and/or CIC and 43 unrelated adult patients with a clinical presentation compatible with LPAC syndrome were included in this present study. For the latter group, we have considered patients presenting with intrahepatic cholelithiasis despite cholecystectomy before 40 years associated with at least one of the following criteria: presence of complications (cholecystitis, cholangitis, or acute pancreatitis), intrahepatic hyperechoic foci with or without sludge or microlithiasis, response to UDCA therapy, and family history.

ABCB4 mutation screening

Total DNA was extracted from whole-blood leukocytes using the Nucleon BACC2 genomic DNA extraction kit (GE Health Care Europe, Amersham, UK). Genomic DNA was amplified with primers specific for the 27 *ABCB4* (NM_018849.2)-coding exons and their intron boundaries. The primer sequences and PCR conditions are available upon request. Mutation was identified using bidirectional DNA sequencing of purified PCR products. Sequences were aligned with Seqscape v5.1 analysis software (Applied Biosystems, Foster City, CA, USA). The *ABCB4* molecular analysis was performed in the Biochemistry and Molecular Genetics Laboratory, Beaujon Hospital, Clichy, France.

ABCB4 multiplex ligation-dependent probe amplification (MLPA) analysis

The *ABCB4* deletion screening was performed by MLPA analysis using the SALSA MLPA kit P109 ABCB4, as recommended in the manufacturer's protocol (MRC-Holland, Amsterdam, The Netherlands). This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the *ABCB4* gene. It contains 22 probes covering *ABCB4*-coding exons, three probes for *ABCB4* promoter, and three probes for the centromeric adjacent *ABCB1* gene. For reference, several probes for other human genes located on different chromosomes are also included. Briefly, four control samples and patient samples (each containing 100 ng of genomic DNA) were used for overnight hybridization with the probe mixes. After ligation and amplification performed with FAM-labeled primers, PCR products were analyzed on an ABI Prism 3130 automatic DNA sequencer (Applied Biosystems). Peak areas for each separated fragment were measured by using Genemapper software v4.0 (Applied Biosystems). Normalized ratios of <0.6 and >1.3 were considered as deletions and duplications, respectively. Ratio profiles between 0.6 and 0.85 were considered as doubtful. DNA samples with *ABCB4* copy number variation were further analyzed by real-time PCR-based gene dosage for confirmation.

Real-time PCR-based gene dosage

We quantified *ABCB4* exons 10 and 11 by determining the threshold cycle (Ct) number at which the increase in the signal associated with exponential growth of PCR products begins. We also quantified the *ALB* gene (encoding albumin) as an endogenous DNA control, and each sample was normalized on the basis of its *ALB* content, as previously described.[38] *ALB* was selected as an endogenous control, because it maps to chromosome 4q11-q13, while *ABCB4* is at chromosome 7q21.1. The relative copy number of the *ABCB4* exon targets was also normalized to a calibrator, consisting of genomic DNA from a normal subject. Final results, expressed as N-fold differences in the *ABCB4* exonic targets copy number relative to the *ALB* gene and the calibrator were determined as follows: $\text{N-fold}=2^{(\Delta\text{Ctsample}-\Delta\text{Ctcalibrator})}$, where ΔCt values of the sample and calibrator are determined by subtracting the average Ct value of the *ABCB4* exon target from the average Ct value of the *ALB* gene. An N-fold value of <0.6 was considered deleted. All PCRs were performed on a LightCycler 480 with the LightCycler 480 SYBR Green I Master kit (Roche Applied Science, Basel, Switzerland). PCR conditions and primer sequences are available on request. Experiments were done with triplicates for each data point.

Microarray characterization of *ABCB4* complete deletions

The practical aspects of array-comparative genomic hybridization (array-CGH) are described in detail elsewhere.[39] Briefly, array-CGH labeling and hybridization were performed on Agilent whole human genome 400K microarrays as recommended in the Agilent manual (Protocol v6.3, October 2010, Agilent Technologies, Palo Alto, CA, USA). Patients' genomic DNA and six pooled normal control DNAs (reference) were labeled with Cy5-dUTP and Cy3-dUTP, respectively. Arrays were scanned with an Agilent DNA Microarray Scanner (G2565BA). Log2 ratios were determined with Agilent Feature Extraction software (v 9.1.3.1). Results were visualized and analyzed with Agilent's Genomic Workbench 5.0 software. DNA sequence information was referred to the public UCSC (University of California Santa Cruz) database (Human Genome Browser, February 2009 assembly: hg 19, National Center for Biotechnology Information (NCBI) Build 37).

Fine characterization of *ABCB4* intragenic deletion breakpoints

Long-range PCR was performed with the Expand 20 kb Plus PCR kit as recommended by the manufacturer (Roche Applied Science). The primer pairs and PCR conditions used to characterize the gross deletions are available upon request. The PCR products were sequenced with the ABI BigDye terminator sequencing kit (Applied Biosystems) on an ABI Prism 3130 automatic DNA sequencer (Applied Biosystems).

RESULTS

In all, 102 index cases with LPAC and ICP/CIC were screened on the basis of their phenotype for mutations in the *ABCB4* gene. A total of 32 *ABCB4* heterozygous loss of function point or short insertion/deletion mutations (including non-sense, frameshift, splice, or previously reported/predicted to be deleterious missense mutations) were identified among the 102 tested index cases: in 37% (16/43) of LPAC patients and in 27% (16/59) of ICP/CIC patients.

The 70 adult patients with no *ABCB4* mutation found (27 with LPAC and 43 with ICP/CIC), were consequently screened for large rearrangements using MLPA. Four heterozygous deletions were identified with this approach in 7% (3/43) of the LPAC patients (BOC propositus of family 1, BEA propositus of family 2, and TIL) and in ~2% (1/59) of the ICP/CIC patients (ROC) (Figure 1). The four deletions were confirmed using real-time PCR-based gene dosage. Clinical features of the individuals with *ABCB4* gene deletions (family 1: patients BOC, ARE, and BOJ; family 2: patient BEA; patient ROC; patient TIL) are summarized in Table 1. Figure 2 shows families 1 and 2 pedigrees including 12 and 5 patients with LPAC syndrome, respectively.

The four *ABCB4* gross deletions were then accurately characterized. They included two whole-gene deletions (TIL and ROC), and two partial intragenic deletions (BEA: exon 10 deletion and BOC: exons 11–19 deletion). Figure 3 shows characteristic array-CGH profiles for the two complete *ABCB4* deletions, as displayed by the workbench software. Deletion of patient ROC is flanked by last non-deleted centromeric probe at position chr7:86 068 686–86 068 745 (numbered as in build 37/hg19 assembly of the NCBI), and telomeric probe at chr7:91 075 851–91 075 910. Deletion of patient TIL is flanked by last non-deleted centromeric probe at chr7:86 907 515–86 907 574 and telomeric probe at chr7:87 246 524–87 246 583. Patient's ROC and TIL deletions were consequently estimated to be ~5 Mb and ~339 kb long, respectively, including 20 and 3 genes in addition to *ABCB4* (Table 1; Supplementary Table 1). The 400K array-CGH data on these two patients have been deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO accession number GSE28676 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28676).

The breakpoints of the two intragenic deletions (patients BEA and BOC) were precisely determined by sequencing the junction long-range PCR products. Only DNA sample from BEA yielded a 3055-bp fragment with primers JONCTIONBEA-U (5′-TGTGACTCGGAC TATGGATTGTT-3′) and JONCTIONBEA-L (5′-CCAAAACTGGATT CACACGCA-3′). Subsequent sequencing revealed that this deletion is in fact a complex rearrangement (Figure 4). A healthy control sample and BEA wild-type allele yielded a 4053-bp non-deleted fragment using the same primers. Only DNA sample from BOC (family 1 propositus) yielded a 2680-bp fragment with primers JONCTIONBOC-U (5′-AAAATAGACCCACTCAGGCAA-3′) and JONCTIONBOC-L (5′-ATGCTACATGCTTATCTAAAACCAT-3′). A healthy control sample yielded no PCR product, the wild-type allele being too large (~27 kb) to be amplified. Sequencing the PCR product revealed a simple deletion of 24 720 bp (Supplementary Figure 1). This deletion was also detected in three other affected individuals with available DNA samples (Figure 2: individuals II:4, II:7, and II:10 of family 1).

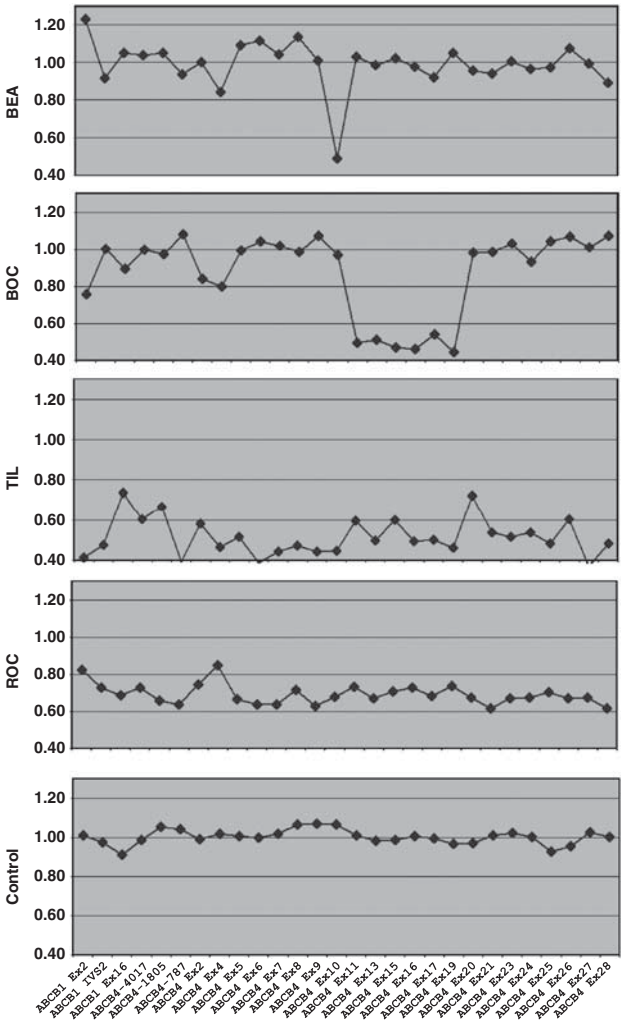

Figure 1 *ABCB4* SALSA MLPA kit P109 peak areas normalized ratio profiles of patients BEA, BOC, TIL, ROC, and one normal control DNA. Two partial intragenic deletions (patients BEA and BOC) and two whole-gene deletions (patients TIL and ROC) were identified. The mean of normalized ratios in patients without deletion was 1.02.

DISCUSSION

In this study, we showed for the first time that a significant subset of patients with symptomatic cholestasis/cholelithiasis has underlying *ABCB4* deletions. Partial or complete heterozygous *ABCB4* deletions were found in 7% of the patients with LPAC and in ~2% of the patients with CIC. A large family of 12 affected patients with severe LPAC and cholecystectomy (family 1) was notably reported (Table 1; Figure 2).

Recent gene dosage methodologies have allowed identification of these *ABCB4* deletions. Our observations urge to systematically test the patients with LPAC for the presence of *ABCB4* deletions. MLPA, a sensitive, rapid, and cost-effective approach seems particularly adapted to routine diagnosis in molecular genetics. We developed a molecular algorithm tailored to *ABCB4* routine analysis that includes *ABCB4* gene dosage by MLPA, in case of *ABCB4* negative sequencing in patients with suggestive phenotype. MLPA allows a fast and inexpensive first-line screening for both the partial and complete *ABCB4* deletions, complementary to a high-resolution technique such as array-CGH that can be used to characterize larger deletions.

Table 1 Clinical and molecular characteristics of the patients carrying gross genomic deletions

Subject	*Gender*	*Age at onset (years)*	*Features at presentation*	*Deletion length*	*Deleted genes*
Family 1			Familial history: see Figure 1	24720 bp	Deletion of *ABCB4* exons 11–19
BOC (propositus)	F	24	LPAC: intrahepatic cholelithiasis with biliary pain. Recurrence (biliary pain and elevated liver enzymes: AST=330 U/l, ALT=416 U/l, and GGT=616 U/) despite cholecystectomy. Free of symptom and normal liver enzymes under UDCA.		
ARE	F	60	Recurrent cholelithiasis despite cholecystectomy. Free of symptom under UDCA.		
BOJ	M	60	LPAC: cholangiopathy with intrahepatic cholelithiasis. Left hepatectomy because of liver atrophy with intrahepatic biliary dilatation and cholecystectomy. Liver biopsy: carcinoma *in situ*, biliary histology abnormalities with ductular proliferation, compatible with *ABCB4* mutation. Free of symptom under UDCA.		
Family 2			Familial history: see Figure 1	998 bp	Deletion of *ABCB4* exon 10
BEA (propositus)	F	30	LPAC: cholelithiasis with biliary pain. Recurrence of biliary symptoms after cholecystectomy. Her mother, maternal grandmother and two maternal aunts had cholecystectomy before the age of 40 (Figure 1).		
Patient TIL	M	36	LPAC with biliary pain. Recurrence (biliary pain and elevated liver enzymes: AST=473 U/l, ALT=722 U/l, and GGT=65 U/l) after cholecystectomy. Free of symptom and normal liver enzymes under UDCA. No evidence of familial history.	~339 kb	4 genes: *P53TG1*, *CROT*, *ABCB4,* and *ABCB1*
Patient ROC	F	26	CIC with biliary pain, jaundice and elevated liver enzymes (AST=1047 U/l, ALT=372 U/l, and GGT=63 U/l). Liver biopsy: biliary histology abnormalities with ductular proliferation and compatible with *ABCB4* mutation. Free of symptom and normal liver enzymes in the absence of oral contraception. Normal pregnancy with delivery at term under UDCA therapy.	~5 Mb	21 genes including *ABCB4* (Supplemental Table 1)

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; U/l, units per liter.
The usual ranges for AST, ALT, and GGT are around 10–35 U/l, 10–45 U/l, and 10–55 U/l, respectively.

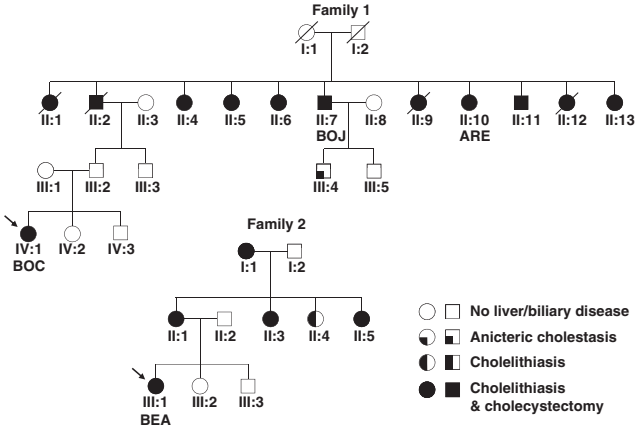

Figure 2 Pedigrees of families 1 and 2. Squares and circles indicate males and females, respectively. Clear symbols indicate unaffected individuals. Arrows indicate propositus. Individuals II:4, II:7, II:10, and IV:1 in family 1 and individual III:1 in family 2 were molecularly tested. Individual II:2 in family 1 was diagnosed with cholangiocarcinoma at 71 years of age.

Real-time PCR-based gene dosage is useful for deletion's confirmation, particularly for the samples with ratio profiles considered as doubtful, that is, between 0.6 and 0.85 (patient ROC; Figure 1).

The two intragenic deletion breakpoints were cloned at the nucleotide level and no recurrent breakpoints were found. That no significant sequence similarity was found between the centromeric and telomeric breakpoints of both the deletions, effectively excluded homologous recombination as the underlying mutational mechanisms in both the cases. However, the presence of microhomology in each of the aberrant junctions is consistent with both the microhomology-dependent replication-based recombination (MMRDR) and non-homologous end-joining (NHEJ) mechanisms.[40] Thus, the complex rearrangement detected in patient BEA can be perfectly explained by either serial replication slippage (SRS, a subpathway of MMRDR) or NHEJ repair of simultaneously generated double-strand breaks (Figure 4), while the simple 24 kb deletion (Supplementary Figure 1) is consistent with a single step of replication slippage (a subpathway of MMRDR), microhomology-mediated break-induced replication (MMBIR, a subpathway of MMRDR) or NHEJ.[40]

In this study, adult patients with LPAC were screened for the *ABCB4* deletions. We assume that *ABCB4* deletions may also be found in children with PFIC3. In some patients with PFIC3, only one *ABCB4* mutation or no mutation has been previously reported while no ABCB4 protein was detected by immunohistochemistry analysis.[19] Some of these genotype–phenotype discrepancies could be explained by the presence of *ABCB4* deletions in the PFIC3 patients. However, in PFIC3 patient with no mutation found, a heterozygous *ABCB4* deletion would not be sufficient to explain the phenotype because PFIC3 is an autosomal recessive disease. Our observations urge to reassess the *ABCB4* molecular analysis in these patients using molecular tools allowing detection of rearrangements. PFIC3 patients who do respond to the UDCA therapy generally have a partial *ABCB4* defect (missense mutations) and the residual phospholipid concentration in the bile combined with UDCA replacement, may be sufficient

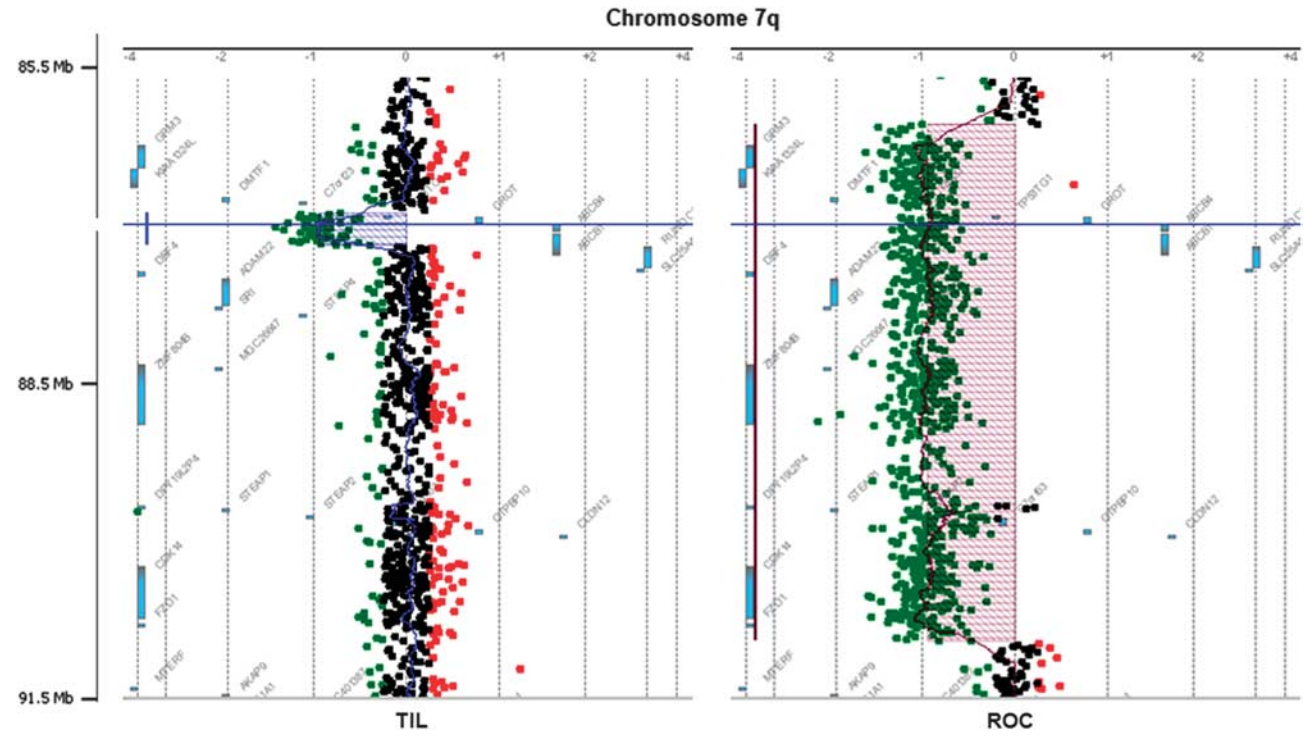

Figure 3 Array-CGH profiles of the two partially characterized *ABCB4* complete deletions: patients TIL and ROC. Zoom on *ABCB4* region at chromosome 7q easily identified the deletions. Horizontal blue line shows *ABCB4* position. Human Genome Browser, February 2009 assembly: hg 19, NCBI Build 37.

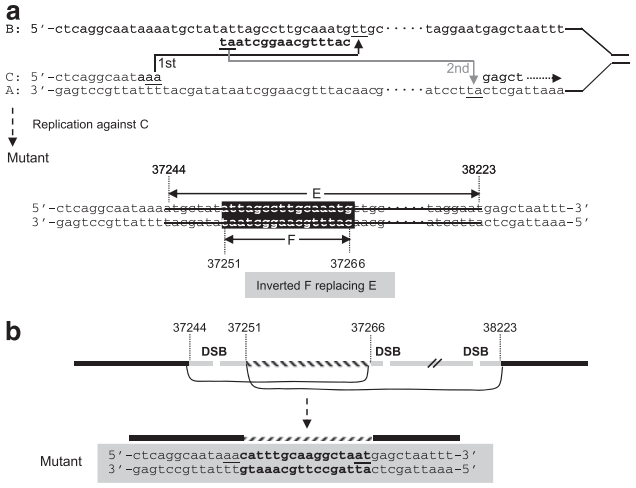

Figure 4 Illustration of two possible mechanisms underlying the complex rearrangement detected in patient BEA. (**a**) In the SRS model,[42,43] the newly synthesized leading strand (C) misaligned with the lagging template strand (B) through inverted short repeats (the first step of slippage); having synthesized a short DNA sequence tract, the C strand dissociated from B and re-annealed to its original template strand (A) via short repeats (the second step of slippage); continued DNA synthesis (right-handed dotted arrow). Then replication against the synthesized strand C resulted in the observed complex rearrangement. (**b**) In the non-homologous end-joining (NHEJ) model, at least two double-strand breaks (DSB) occurred within the sequences deleted. One short internal sequence was re-captured in an inverted orientation during the NHEJ repair. Numbers indicating nucleotide positions are in accordance with GenBank accession EF034088.1. Short repeats are underlined.

to reduce bile salt toxicity below a critical threshold.[19] *ABCB4* genotyping (including deletion's detection) should help to select those PFIC3 patients who could benefit from the UDCA therapy.

In clinical practice, the establishment of a comprehensive *ABCB4* alteration-screening algorithm will permit the use of *ABCB4* genotyping, to confirm the diagnosis of LPAC syndrome in young adults who present with a symptomatic cholelithiasis and allow familial testing. One argument in support of molecular testing for *ABCB4* deletion is benefit of the UDCA therapy of both the symptomatic and asymptomatic cholelithiasis in patients with *ABCB4* deficiency.[18] Depending on the results, long-term curative or prophylactic UDCA therapy could be initiated early to prevent the occurrence or recurrence of syndromes and their potential severe complications. Patient ROC who suffered from CIC may also be susceptible to the development of ICP that carries a risk of premature delivery and sudden fetal death. Identification of the *ABCB4* deletions may also benefit patients with CIC and ICP, as UDCA is recommended to reduce pruritus, and probably prematurity without adverse side effects.[41] Management of ICP also includes close monitoring and early delivery for the fetus. All the patients with *ABCB4* defect in this study have benefited from the UDCA treatment. These observations are remarkable examples of the inter-relationship between molecular biology and clinical medicine.

The extreme variability and the wide spectrum of *ABCB4* alteration-related phenotypes make genotype–phenotype correlations difficult, although they are of crucial importance for the patients and their families. Further comparative studies of patients with well-characterized genotypes (including deletions) and phenotypes will help determine whether *ABCB4* mutation types influence clinical outcomes. Three of the four patients with deletions (BEA, BOC, and TIL) developed LPAC that recurred despite cholecystectomy. Patient ROC presented a very large *de novo* ~5 Mb deletion encompassing the *ABCB4* locus and 20 additional genes including *ABCB1* gene (Supplementary Table 1). Surprisingly, this patient presented a less severe phenotype only consisting in CIC, while being haploinsufficient for 21 genes. Unlike the *ABCB4* gene, none of these 20 genes is known

to be associated with an inherited human disease according to the OMIM database (http://www.ncbi.nlm.nih.gov/omim). Variable expressions of the liver diseases caused by *ABCB4* mutations have previously been reported. Comorbidity factors, environmental influences, or unknown genetic modifiers may modulate these phenotypes.[13,19] Our observations reinforce the potential existence of these genetic modifiers.

Gene dosage technologies have allowed the identification of *ABCB4* deletions in a significant subset (7%) of patient with LPAC syndrome. An early diagnosis of this biliary disease would be beneficial because of the potential preventive effect of UDCA on the biliary complications. These data must now be taken into account in patient diagnosis and follow-up.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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