**Name of journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 3796**

**Columns: BRIEF ARTICLE**

***ABCB4* mutations underlie hormonal cholestasis but not pediatric idiopathic gallstones**

Jirsa M *et al. ABCB4* mutations and pediatric gallstones

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**Author contributions:** Jirsa M and Hřebíček M designed the study, wrote the draft and performed statistical analysis, Bronský J and Nevoral J selected patients with idiopathic gallstones, Dvořáková L supervised mutation analysis and performed pathogenicity predictions, Šperl J, Šmajstrla V and Horák J provided clinical data and samples of the LPAC families. All contributed to writing the draft.

**Supported by** The project (Ministry of Health, Czech Republic) for development of research organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support; PRVOUK-P24/LF1/3 and MH CZ – DRO VFN64165 to Dvořáková L and Hřebíček M

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**Received:** May 23, 2013  **Revised:** July 25, 2013

**Accepted:** August 16, 2013

**Published online:**

**Abstract**

**Aim:** To investigate contribution of *ABCB4* mutations to pediatric idiopathic gallstone disease and the potential of hormonal contraceptives to prompt clinical manifestations of multidrug resistance protein 3 deficiency.

**Methods:**Mutational analysis of*ABCB4,* screening for copy number variations by multiplex ligation-dependent probe amplification, genotyping for low expression allele c.1331T>C of *ABCB11* andgenotyping for variation c.55G>C in *ABCG8* previouslyassociated with cholesterol gallstones in adults was performed in 35 pediatric subjects with idiopathic gallstones who fulfilled the clinical criteria for low phospholipid-associated cholelithiasis syndrome (LPAC, OMIM #600803) and in 5 young females with suspected LPAC and their families (5 probands, 15 additional family members). The probands came to medical attention for contraceptive-associated intrahepatic cholestasis.

**Results:** A possibly pathogenic variant of *ABCB4* was found only in one of the 35 pediatric subjects with idiopathic cholesterol gallstones whereas 15 members of the studied 5 LPAC kindreds were confirmed and another one was highly suspected to carry predictably pathogenic mutations in *ABCB4*. Among these 16, however, none developed gallstones in childhood. In 5 index patients, all young females carrying at least one pathogenic mutation in one allele of *ABCB4*, manifestation of LPAC as intrahepatic cholestasis with elevated serum activity of gamma-glutamyltransferase was induced by hormonal contraceptives. Variants *ABCB11* c.1331T>C and *ABCG8* c.55G>C were not significantly overrepresented in the 35 examined patients with suspect LPAC.

**Conclusion:** Clinical criteria for LPAC syndrome caused by mutations in *ABCB4* cannot be applied to pediatric patients with idiopathic gallstones. Sexual immaturity even prevents manifestation of LPAC.

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**Key words:** Idiopathic cholelithiasis; Intrahepatic cholestasis; Oral contraceptives; Low phospholipid-associated cholelithiasis; Gallbladder disease 1

**Core tip:** Mutations in *ABCB4* are not overrepresented in children with idiopathic gallstones who fulfill the clinical and laboratory criteria for low phospholipid-associated cholelithiasis syndrome (Gallbladder Disease 1, OMIM #600803); Sexual immaturity prevents manifestation of low phospholipid-associated cholelithiasis; In young females, manifestation of low phospholipid-associated cholelithiasis syndrome such as intrahepatic cholestasis with elevated serum activity of gamma-glutamyltransferase may be induced by hormonal contraceptives.

Jirsa M, Bronský J, Dvořáková L, Šperl J, Šmajstrla V, Horák J, Nevoral J, Hřebíček M. *ABCB4* mutations underlie hormonal cholestasis but not pediatric idiopathic gallstones.

**Available from:**

**DOI:INTRODUCTION**

Low phospholipid-associated cholelithiasis syndrome (LPAC, synonym Gallbladder disease 1, OMIM #600803) has been defined as symptomatic and recurring cholelithiasis associated with mutations in *ABCB4* encoding multidrug resistance protein 3 (MDR3), the canalicular phospholipid export pump[1, 2]. LPAC should be suspected in patients with symptomatic cholelithiasis in whom at least one minor criterion is present. These minor criteria are proposed as: (1) age below 40 years at the onset of symptoms; (2) recurrence after cholecystectomy; (3) intrahepatic hyperechoic foci with a topography compatible with lipid deposits along the luminal surface of the intrahepatic biliary tree; (4) intrahepatic sludge; (5) microlithiasis; (6) history of gallstones in first-degree relatives; or (7) history of intrahepatic cholestasis of pregnancy[3]. The distribution of associated *ABCB4* mutations in conserved regions of the gene, as well as their type, strongly support the role of partial MDR3 deficiency in LPAC, with decreased MDR3 activity and/or expression altering biliary lipid composition.

Apart from LPAC, mutations in *ABCB4* that reduce but do not abrogate the activity of MDR3 can cause a variety of milder forms of familial intrahepatic cholestasis type 3 (OMIM #602347), with slowly progressive or non-progressive hepatobiliary disease or anicteric cholestasis with varying liver fibrosis in adulthood[4]. Several reports[5-7] have shown that intrahepatic cholestasis of pregnancy is associated with *ABCB4* mutations in some women. Finally, that contraceptive-induced cholestasis (CIC) may be associated with mutations in *ABCB4* has also been proposed: Asymptomatic gallstones and clinically silent cirrhosis, diagnosed later as progressive familial intrahepatic cholestasis type 3, became manifest in a 17-year-old girl when cholestasis developed on ingestion of contraceptive pills containing ethinylestradiol, 30 g, and levonorgesterol, 150 g[8], and isolated gallstone disease unmasked by oral contraception and associated with *ABCB4* mutation has been reported[1]. In contrast, no mutations in *ABCB4* were found in 5 subjects with CIC studied by Lang et al.[9].

In our previous study[10] we focused on the role of the common variants c.523A>G (p.Thr175Ala) and c.1954A>G (p.Arg652Gly) in *ABCB4*, c.1331T>C (p.Val444Ala) in *ABCB11* and c.55 G>C (p.Asp19His) in *ABCG8* in pediatric gallstone disease. These variants are considered either as potentially pathogenic or as susceptibility alleles for cholesterol cholelithiasis in adults; however, they were not observed to contribute to genetic predisposition to gallstones in childhood[10].

In this study we investigated: (1) the role of *ABCB4* mutations in the etiology of pediatric idiopathic gallstones; and (2) the capability of hormonal contraceptives to unmask hitherto clinically silent MDR3 deficiency.

**MATERIALS AND METHODS**

***Pediatric patients with gallstones***

Pediatric patients with gallstones were selected as described[10] (see Figure 1 for the selection algorithm)*.* Briefly, one hundred and nine children (53 males and 56 females) with gallbladder gallstones who had been hospitalized at the Department of Pediatrics, Faculty Hospital Motol, Prague, between 1995-2004, were considered. In 22 patients, gallstones were clearly associated with another disease such as Down syndrome, Gaucher disease, cystic fibrosis, hemolytic anemia, inflammatory bowel disease, immune deficiency and Gilbert syndrome. Thirty-three of the 87 invited patients did not respond. In 13 of 54 patients, the etiology of gallstones was uncertain. However, as these 13 patients had at least one the following: long-term parenteral nutrition, treatment with cephalosporins or furosemide, dyslipidemia, hepatobiliary infectious disease or obesity (BMI > 27), e.g. conditions that could promote gallstone formation, they were not enrolled. In 41 patients, gallstones were most likely idiopathic. For *ABCB4* mutation testing, only 35 of these 41 patients (including only one of the monozygous twins) with idiopathic gallstones were selected who had at least one parent or grandparent with gallstones. These subjects (15 males and 20 females with positive family history), all unrelated Caucasians of Czech origin, met the major criterion and minor criteria (a) and (f) of Rosmorduc and Poupon[3]. The mean age at the diagnosis of cholelithiasis was 10.7 ± 5.0 years (range 1-17). Nineteen of these 35 patients (13 girls and 6 boys) underwent cholecystectomy with no recurrence after surgery. As of this writing, all cholecystectomized patients are well, without abdominal pain or jaundice.

***Young adults with suspect LPAC***

Five young adult female patients with symptomatic gallstones (age below 40 years at the onset of symptoms), a history of intrahepatic cholestasis, and a family history of gallstones in first-degree relatives were referred for *ABCB4* analysis. Their clinical characteristics are summarized in Table 1*.* None of the patients had hyperechoic foci in the liver parenchyma or proven intrahepatic sludge; duodenal bile was not investigated for microlithiasis. Nonethless, all met the proposed criteria for LPAC[3]. In patient I, intrahepatic cholestasis associated with exposure to an oral contraceptive containing ethinylestradiol, 0.030 mg, and levonorgestrel, 0.125 mg (Minisiston; Jenapharm, Jena, Germany) was the first clinical symptom of LPAC. Cholestasis resolved rapidly after withdrawal of the contraceptive. However, the patient developed cholecystolithiasis within one year despite ursodeoxycholic acid administration (15 mg/kg) and underwent cholecystectomy. One year later, ursodeoxycholic acid was withdrawn because the patient was completely asymptomatic with normal clinical-laboratory test results. Rechallenge with another oral contraceptive containing ethinylestradiol, 0.020 mg, and desogestrel, 0.150 mg (Mercilon; Organon, Oss, The Netherlands) two years after cholecystectomy was followed within several weeks by a second attack of cholestasis. Clinical and laboratory findings improved again rapidly when the medication was withdrawn. None of the other four index patients mentioned any problems associated with the use of contraceptives; the data on contraceptives presented in Table 1 were obtained in part from clinical records and in part by specific questioning.

The patient studies were approved by the Institutional Review Board of the Faculty Hospital Motol. Either both parents or the examined subjects, when aged over 15 years, gave written informed consent before blood sampling.

***Mutational analysis***

Twenty-seven fragments covering all exonic (protein-coding) regions of *ABCB4* and including portions of adjacent intronic sequences were amplified from genomic DNA by PCR (primer sequences are available from the corresponding author). The DNA sequence of purified PCR products was analyzed on an ABI-PRISM 3100-Avant automated DNA sequencer (Applied Biosystems, Foster City, CA). Ensembl Acc. No. ENSG00000005471 and GenBank Acc. No. NM\_018849.2 served as genomic and cDNA reference sequences. Mutations found by DNA sequencing were independently confirmed by restriction fragment length polymorphism analysis after digestion of the corresponding PCR product with restriction enzymes. In addition, *ABCB4* was scanned for deletions/duplications by multiplex ligation-dependent probe amplification, using SALSA MLPA KIT P109 *ABCB4* (MRC-Holland, Amsterdam, The Netherlands) according to manufacturer’s instructions.

The low expression allele c.1331T>C of *ABCB11*[11] was detected as the presence of a PCR-*Bsu*RI restriction fragment length polymorphism. The variation c.55G>C in *ABCG8* associated with cholesterol gallstones in adults[12] was detected as described by Hubáček *et al*[13]. Pathogenicity of missense variations was predicted *in silico* by SIFT[14], PMut[15], PolyPhen-2[16] and MutationTaster[17].

***Statistical analysis***

The data are presented as mean and standard deviation, or as frequencies when appropriate. We used chi-square testing to check whether genotype frequencies were consistent with Hardy-Weinberg equilibrium. Differences between genotype frequencies were analyzed by two-sided Fisher exact testing, using the approximation of Katz, with the InStat3 program (GraphPad Software, La Jolla, CA).

**RESULTS**

***Pediatric patients with gallstones***

In the group of pediatric patients with idiopathic gallstones selected for genetic examination, analysis of protein-coding exons and intron/exon junctions of *ABCB4* identified no obvious pathogenic mutations. In patient 31, a novel heterozygous variation was found (c.2222C>T, leading to predicted conservative amino acid substitution p.Pro741Leu in the extracellular loop between transmembrane domains 7 and 8). The substitution was rated as neutral by all four pathogenicity prediction programs. Another predicted amino acid substitution (p.Gly773Val, localized in transmembrane domain 8 and caused by the novel mutation c.2318G>T) was found in heterozygous state in patient 32. This conservative substitution was rated as disease-causing by MutationTaster, possibly pathogenic by PolyPhen-2, and neutral by SIFT and PMut. In addition, 6 known coding (5 synonymous) and 6 known non-coding variations were found (Table 2). None of these changes is reportedly associated with hepatobiliary disease, with the possible exception of c.1954A>G (p.Arg652Gly), found previously in heterozygous state in subjects 4, 8 and 26[10]. However, the c.1954A>G variant was not overrepresented (3/70, allelic frequency 0.043) in our patients as compared with a healthy adult Czech Caucasian population (allelic frequency 0.090, 27 heterozygotes in 150 controls, OR = 0.48, CI: 0.16-1.48, *P* = 0.17).

***Families with suspect LPAC***

Two of the five probands carried a single heterozygous nonsense mutation, two were heterozygotes for the missense mutation c.523A>G (p.Thr175Ala, rs58238559), and one was a compound heterozygote for the same missense mutation (c.523A>G) and for the frameshift mutation c.1371delG (p.Gln458Argfs\*7) (Figure 2). The variation c.523A>G was found on 30% (3/10) of alleles in patients with LPAC, whereas only 2.7% of control alleles from the Czech population carried guanine at the position 523 (8/300, 8 heterozygotes in 150 control individuals, OR = 8.00, CI: 2.20-29.24, *P* = 0.012). While the number of patients was too low to make the result fully convincing, this observation suggests that p.Thr175Ala at least confers susceptibility to hepatobiliary disease. All three null mutations were novel to our best knowledge.

No deletions/duplications in *ABCB4* were detected in index patients by multiplex ligation-dependent probe amplification.

Two probands were homozygous and the other three probands were heterozygous for the low-expression *ABCB11* variant c.1331T>C (p.Val444Ala) (Figure 2). One proband had a c.55G|C genotype while four other probands were homozygous for the wildtype allele c.55G in *ABCG8.* To assess the segregation of the genotype and phenotype in the families of all index patients, first degree relatives were examined. As can be seen from the family trees depicted in Figure 2, the parents in families I, II, IV, and V who carry the same mutation as the probands in a heterozygous state were symptomatic. This indicates that the null mutations in families I - III and even the missense mutation leading to p.Thr175Ala in families IV and V all are likely sufficient in a heterozygous state to promote the LPAC phenotype. In contrast, variations *ABCB11* c.1331T>C and *ABCG8* c.55G>C, found in probands and 11 family members carrying mutations in *ABCB4* do not seem to affect the penetrance of LPAC (Figure 2).

**DISCUSSION**

The only possible pathogenic mutation in *ABCB4* found in pediatric patients with idiopathic gallstones who met clinical criteria for the diagnosis of LPAC was the variation c.2318G>T (p.Gly773Val ) found in heterozygous state in only one affected subject. The nucleotide change c.1954A>G found in 3 other pediatric gallstone subjects is common in the European, Caucasian, and African general population, but it also has been found in a patient with LPAC and low biliary phospholipid in whom its predicted consequence p.Arg652Gly was hypothesized to be conditionally penetrant, leading to clinical symptoms only under certain circumstances, such as pregnancy, or when combined with another mutation[18]. In contrast, no correlation of the *ABCB4* genotype c.1954A|G with MDR3 expression level in the liver, as measured by Western blot, was observed in a study by Meier et al.[11] and the substitution was rated as neutral by all software tools used. Our finding that the genotype c.1954A|G was neither overrepresented nor significantly underrepresented in patients with gallstones may indicate the negligible role of this variation in etiology of pediatric idiopathic gallstones. Similar conclusions could be drawn for both carriers and homozygotes for the low expression variant of bile salt export pump and for the carriers of the *ABCG8* variation c.55G>C.

Interestingly, the *ABCB4* variation c.523A>G (p.Thr175Ala), found in three index patients with LPAC, was not present in any of our patients with pediatric gallstones. The allele c.523G is linked to cholestatic disease[1] although it also is found in healthy Caucasian populations at an allelic frequency of 0.025-0.032[9, 19, 20]. The threonine residue at position 175 is highly conserved, lying in a Thr-Arg-Leu-Thr cluster required for MDR3 adenosine triphosphatase (ATPase) activity. While the functional consequences of replacement of threonine at position 175 by a neutral amino-acid residue having a hydrophobic side chain were not evaluated in MDR3, they were studied in yeast in the close homologue P-glycoprotein[21], in which the substitution p.Thr169Ile resulted in a complete loss of substrate-induced P-glycoprotein ATPase activity. The substitution p.Thr175Ala, predicted uniformly to impair protein function by SIFT, PMut, PolyPhen-2 and MutationTaster is thus considered a disease-associated mutation[3] with incomplete penetrance.

Neither the 14 confirmed and one suspected heterozygous carriers of *ABCB4* mutations investigated in the second part of our study nor the heterozygotes reported previously by others[1, 2, 4, 22-24] developed symptomatic gallstones without progressive familial intrahepatic cholestasis in childhood. This suggests that other pathogeneses of idiopathic gallstones in childhood should be sought. Since we did not assay phospholipid and cholesterol concentrations in bile from our 35 pediatric subjects, we cannot definitively claim that they did not have LPAC; only that, if they had LPAC, it was associated neither with demonstrable *ABCB4* mutation (this study) nor with the studied variations in *ABCB11* and *ABCG8*[10]. We suggest that to carry out *ABCB4* sequencing in pediatric patients with idiopathic cholesterol gallstones who meet only some of the present criteria for assigning the diagnosis of LPAC may be unproductive. We believe that the validity of these criteria for LPAC associated with *ABCB4* mutation should be re-assessed, in pediatric patients at least, and propose that the present criteria at this juncture be considered to apply only to adults aged less than 40 years.

The observations that LPAC syndrome becomes manifest after middle adolescence and that young females heterozygous for pathogenic mutations in *ABCB4* developed CIC and/or manifested previously asymptomatic gallstones during administration of combined oral contraceptives are most likely explained by known changes in biliary lipid composition during the second decade of life. Gallstones hardly ever occur in children, but are frequent in adults; this difference seems to be due to the low concentrations of cholesterol in the bile of children[25]. Children have reduced biliary cholesterol : bile salt excretion ratios[26]. Therefore, even at low rates of phospholipid secretion caused by incomplete MDR3 deficiency, bile is not saturated with cholesterol. The known increase in the biliary cholesterol saturation index in young adults[26], together with the decreased biliary secretion rate of phosphatidylcholine in carriers of mutations in *ABCB4*, shifts the cholesterol-solubility equilibrium to the borderline. Even the low load of exogenous hormones contained in contraceptives or other hormonally active drugs, which inhibit bile salt secretion[27] and further decrease secretion of phospholipids into bile proportionally to bile salt flow[28], can then precipitate cholestasis and promote cholesterol crystallization from supersaturated bile, with formation of intrahepatic sludge and of gallstones.

A practical question can be raised on the safety of contraceptives in women with MDR3 deficiency. Our patients heterozygous for null mutations developed CIC rapidly and consequently contraceptives had to be withdrawn. In contrast, two patients heterozygous for the missense MDR3 variant p.Thr175Ala tolerated long-term administration of oral contraceptives after cholecystectomy without apparent worsening in hepatobiliary disease. Interestingly, patient V reported pruritus and her serum levels of GGT were repeatedly increased twofold when she used estrogen-free intrauterine contraception. We therefore believe that the heterozygous state for missense mutations in *ABCB4* is not an *a priori* contraindication to oral contraception. However, monitoring of clinical status and clinical-laboratory indices of hepatobiliary injury is essential in such cases.

In conclusion, our findings indicate that clinical criteria for LPAC caused by mutations in *ABCB4* cannot be applied to pediatric patients with idiopathic gallstones. Sexual immaturity prevents manifestation of LPAC even in carriers of pathogenic mutations in *ABCB4.*  In young females, manifestation of LPAC as intrahepatic cholestasis with elevated serum activity of gamma-glutamyltransferase may be triggered by hormonal contraceptives.

**ACKNOWLEDGEMENTS**

We thank A S Knisely, Institute of Liver Studies, King's College Hospital, London, UK, for comments on the manuscript, and Lucie Budišová, IKEM, and Michaela Boučková, Institute of Inherited Metabolic Diseases, both Prague, Czech Republic, for technical assistance.

**COMMENTS**

***Background***

Mutations in *ABCB4*, the variation c.55G>C in *ABCG8* and the low expression allele c.1331T>C of *ABCB11* may affect biliary lipid composition and increase saturation of bile with cholesterol. Mutations in *ABCB4* are known to cause low phospholipid-associated cholelithiasis (LPAC) in young adults. The variation c.55G>C in *ABCG8* has been linked with gallstones in adults.

***Research frontiers***

Since saturation of bile with cholesterol in children is lower than in adults, the authors anticipated a strong contribution of the above listed genetic variations to pediatric idiopathic gallstones and conducted a genetic study in pediatric LPAC-like gallstone patients and in young adults with suspect LPAC who came to medical attention due to contraceptive-induced cholestasis. Whereas young adult females with clinically defined LPAC carried mutations in *ABCB4*, no association with the studied variants was found in pediatric LPAC-like subjects.

***Innovations and breakthroughs***

Sexual immaturity prevents manifestation of LPAC even in carriers of pathogenic mutations in *ABCB4.*

***Applications***

Clinical criteria for LPAC caused by mutations in *ABCB4* cannot be applied to pediatric patients with idiopathic gallstones and to carry out *ABCB4* sequencing in pediatric patients with idiopathic cholesterol gallstones may be unproductive. Heterozygous state for some missense mutations in *ABCB4* is not an *a priori* contraindication to oral contraception; however, monitoring of clinical status and clinical-laboratory indices of hepatobiliary injury is essential in such cases.

***Terminology***

Biliary lipid secretion is mediated by three ABC transporters: *ABCB11* encodes the bile salt export pump, *ABCB4* encodes the canalicular lecithin pump MDR3 (multidrug resistance protein 3) and the genes *ABCG5* and *ABCBG8* encode two proteins named sterolins which form a heterodimeric ABC transporter responsible for biliary secretion of cholesterol and plant sterols.

***Peer review***

It is an excellent manuscript submitted to reevaluate the criteria for LAPC associated with *ABCB4* mutation and provide the data of both pediatric idiopathic gallstone and young women with LAPC hormonal cholestasis by oral contraceptives. The mechanism of cholelithiasis formation with *ABCB11* and *ABCG8* mutation was also observed. And 5 probands with the detail pedigrees were proved. Ethics of the research was given by written informed consent.

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**P-Reviewer** Han TQ  **S-Editor** Wen LL  **L-Editor**  **E-Editor**

**Figure 1Flowchart describing the algorithm for selection of pediatric patients with low phospholipid-associated cholelithiasis syndrome-like idiopathic gallbladder gallstones.**

**Figure 2Family trees of the five unrelated female probands with low phospholipid-associated cholelithiasis syndrome syndrome and *ABCB4* mutations.** Phenotypeof low phospholipid-associated cholelithiasis syndrome (LPAC) syndrome is indicated with gray asterisks, index patients are marked with arrows. Genotypes for *ABCB11* c.1331T>C and *ABCG8* c.55G>C are shown at the genealogical symbols. n. a. – DNA not available. The father´s brother of the proband **I** (F2-1) aged 53 years had no signs of LPAC. The proband´s grandfather (F1-1) developed gallstones after 40 years of age. Similarly, the father of proband III (F1-1) experienced his first attack of biliary pain at the age of 60 years. His DNA sample was not available for analysis; however, he is most likely a heterozygous carrier of c.523A>G (p.Thr175Ala) as depicted. The children (F3-1 and F3-2) of proband III are nine years old twins with no signs of LPAC. The DNA of the sister of proband IV (F2-1), who met the clinical criteria of LPAC, was analyzed for *ABCB4* mutations with negative results.

**Table 1 Characteristics of young women with low phospholipid-associated cholelithiasis syndrome and proven *ABCB4* mutations**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Patient ID** | **Year of birth** | **Age at cholecystectomy (yr)** | **Age at liver biopsy**  **(yr)** | **Pregnancies**  **(yr)** | **Contraceptives and complications**  **(yr)** |
| I | 1980 | 20 | 19, periportal fibrosis | Nulliparous | 18 Minisiston, withdrawn for CIC  22 Mercilon, withdrawn for CIC |
| II | 1978 | 17 | 21, periportal fibrosis | Nulliparous | 18 Tri-regol, withdrawn for CIC |
| III | 1973 | 22 | 22, periportal fibrosis | 30, with ICP | 20-22 Cilest, withdrawn for CIC |
| IV | 1967 | 28 | 31, normal histology | 19, without ICP | 28 Tri-regol, withdrawn for CIC  39-now Lunafem, tolerated |
| V | 1973 | 20 | 20, periportal fibrosis | 23, without ICP  32, without ICP | 19-?, withdrawn for CIC  24-31 Marvelon, tolerated  32-now Mirena, permanent pruritus,  GGT twice normal |

Chemical composition of contraceptives: (1) Minisiston (Jenapharm, Jena, Germany): ethinylestradiol, 0.030 mg; levonorgestrel, 0.125 mg; (2) Mercilon (Organon, Oss, The Netherlands): ethinylestradiol, 0.020 mg; desogestrel, 0.150 mg; (3) Tri-regol (Chemical Works of Gedeon Richter, Budapest, Hungary): ethinylestradiol, 0.030 – 0.040 – 0.030 mg; levonorgestrel, 0.050 – 0.075 – 0.125 mg; (4) Cilest (Janssen Pharmaceutica, Beerse, Belgium): ethinylestradiol, 0.035 – 0.035 – 0 mg, norgestimate, 0.250 – 0.250 – 0 mg; (5) Lunafem (Bayer Schering Pharma, Berlin, Germany): ethinylestradiol, 0.020 mg; gestodene, 0.075 mg; (6) Marvelon (Organon, Oss, The Netherlands): ethinylestradiol, 0.030 mg; desogestrel, 0.150 mg; (7) Mirena (Bayer Schering Pharma, Berlin, Germany): levonorgestrel, 0.020 mg. Estrogen free intrauterine application. CIC: Contraceptive-induced cholestasis.

**Table 2****Known variations in *ABCB4*, *ABCB11* and *ABCG8* found in 35 pediatric subjects with idiopathic gallstones***.*

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient. ID** | **Variations in the coding sequence of *ABCB4*** | | | | | | ***ABCB11* low expression allele** | ***ABCG8***  **variation** |
|
|  | c.147C>T | c.175C>T | c.459T>C | c.504C>T | c.711A>T | c.1954A>G | c.1331T>C | c.55G>C |
|  | Ser49Ser | Leu59Leu | Phe153Phe | Asn168Asn | Ile237Ile | Arg652Gly | Val444Ala | Asp19His |
|  | [rs8187789](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=8187789) | [rs2302387](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2302387) | [rs2230027](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2230027) | [rs1202283](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1202283) | [rs2109505](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2109505) | [rs2230028](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2230028) | [rs2287622](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2287622) | [rs11887534](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=11887534) |
| 1 | CC | CC | TT | TT | AA | AA | CC | GG |
| 2 | CC | CC | TT | CT | AA | AA | TC | GG |
| 3 | CC | CC | TT | CT | AA | AA | TC | GG |
| 4 | CC | CC | TT | CT | AT | AG | TC | GG |
| 5 | CC | CC | TT | TT | AA | AA | CC | GG |
| 6 | CC | CC | TT | CC | AA | AA | TC | GG |
| 7 | CC | CC | TT | CT | AA | AA | TC | GG |
| 8 | CC | CC | TT | CC | AA | AG | TC | GG |
| 9 | CC | CC | TT | TT | AA | AA | TT | GG |
| 10 | CC | CC | TT | TT | AA | AA | CC | GG |
| 11 | CC | CC | TT | TT | AA | AA | TC | GG |
| 12 | CC | CC | TT | TT | AA | AA | CC | GG |
| 13 | CC | CC | TT | CT | AA | AA | TC | GG |
| 14 | CC | CC | TT | CC | AA | AA | CC | GG |
| 15 | CC | CC | TT | TT | AA | AA | TC | GC |
| 16 | CC | CC | TT | CC | AA | AA | TC | GC |
| 17 | CC | CC | TT | CC | AA | AA | TC | GG |
| 18 | CC | CC | TT | TT | AA | AA | TC | GC |
| 19 | CC | CC | TT | CT | AA | AA | TC | GC |
| 20 | CC | CC | TT | CT | AA | AA | TT | GG |
| 21 | CC | CC | TT | TT | AA | AA | TC | GG |
| 22 | CC | CC | TT | CT | AA | AA | TT | GG |
| 23 | CC | CC | TT | CT | AA | AA | TT | GG |
| 24 | CC | CT | TT | CC | AT | AA | CC | GG |
| 25 | CC | CC | TT | CC | AA | AA | TC | GG |
| 26 | CT | CT | TC | CC | AA | AG | TC | GG |
| 27 | CC | CC | TT | TT | AA | AA | TC | GG |
| 28 | CC | CC | TT | TT | AA | AA | TT | GG |
| 29 | CC | CC | TT | CT | AA | AA | TT | GG |
| 30 | CC | CC | TT | TT | AA | AA | CC | GC |
| 31 | CC | CC | TT | CT | AA | AA | TT | GC |
| 32 | CC | CC | TT | CT | AA | AA | TT | GG |
| 33 | CC | CC | TT | TT | AA | AA | TC | GG |
| 34 | CC | CC | TT | CC | AA | AA | TT | GG |
| 35 | CC | CC | TT | TT | AA | AA | CC | GG |
| Allelic frequency of variant alleles in patients with gallstones, HapMap populations and Czech population controls | | | | | | | | |
| Allele | T | T | C | T | T | G | C | G |
| Gallstone patients | 0.014 | 0.029 | 0.014 | 0.571 | 0.029 | 0.043 | 0.471 | 0.086 |
| HapMap CEU | 0 | 0.112 | 01 | 0.664 | 0.175 | 0.075 | 0.408 | 0.085 |
| HapMap HCB | 0 | 0.167 | 01 | 0.344 | 0.222 | 0.023 | 0.333 | 0.022 |
| HapMap JPT | 0 | 0.273 | 01 | 0.442 | 0.300 | 0.023 | 0.261 | 0.011 |
| HapMap YRI | 0.042 | 0.525 | 0.11 | 0 | 0.362 | 0.392 | 0.425 | 0.042 |
| Czech controls | n.d. | n.d. | n.d. | n.d. | n.d. | 0.090 | 0.400 | 0.0672 |

1Results from corresponding populations studied in Environmental Genome Project (NIEHS ES15478 project). HapMap population data were not available for this variation; 2Frequency in 285 Czech controls[15]. n.d.: Not done.