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The role of ABC transporters' gene polymorphism in the etiology of intrahepatic cholestasis of pregnancy

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Objectives: The etiology of intrahepatic cholestasis of pregnancy (ICP) involves environmental, hormonal and genetic factors. It is thought that ICP may be related to the polymorphic variants of several genes involved in the metabolism and transport of bile acids (BA). The goal of our study was to evaluate the possible role of genetic polymorphic variants of ABC transporters in patients with ICP.

Material and methods: 96 women with ICP (mean age of 30.42 years, mean gestational age of 36.83 gestation weeks) and 211 healthy pregnant women (mean age of 30.68 years, mean gestational age of 39.05 gestation weeks) were enrolled in the study. Genetic analysis was performed using a polymerase chain reaction / restriction fragment length polymorphism (PCR/RFLP) method. The following polymorphisms were analysed: 1331T > C (V444A) ABCB11 and 1954A > G (R652G) ABCB4.

Results: Our analysis of frequency of genotypes and alleles of the 1954A > G ABCB4 polymorphism revealed no significant differences between the ICP and control groups. For the 1331T > C polymorphism of the ABCB11 gene the results revealed a higher frequency of 1331CC genotypes in the ICP group (39.58% vs. 29.38%. OR = 1.57, p = 0.05). Also, the frequency of the 1331C allele was higher in the ICP group compared to the control group (64.06% vs. 55.69%, OR = 1.42, p = 0.03).

Conclusions: The overrepresentation of mutated variants of the 1331T > C ABCB11 polymorphism in the ICP group suggests its contribution to the etiology of the intrahepatic cholestasis of pregnancy. Analysis of genotypes' co-existence pointed to the possibility of the mutated variants of polymorphism 1954A > G ABCB4 and 1331T > C ABCB11 having a summation effect on the development of ICP.

Key words: intrahepatic cholestasis of pregnancy, ICP, bile acid, ABC transporter, polymorphism

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is an obstetrical complication, which affects about 1.5% of pregnancies among Caucasians. The condition is quite serious since it may lead to intrauterine fetal demise. The relevant process behind ICP is the excessive accumulation of bile acids in hepatocytes and the inhibition of bile excretion [1]. Overproduction of bile acids or their impaired removal from hepatocytes may be the reason of this pathology, which leads to increased bile acid serum concentration [2–4].

Some of the studies show that polymorphic variants of genes encoding ABC transporting proteins may play a role in the etiology of ICP. An ABCB11 gene encodes an ABC transporter named Bile Salt Export Pump (BSEP) that is responsible for the removal of bile acids from hepatocytes [5]. The degree of the impairment of bile acid transport by BSEP depends on the polymorphic variants of ABCB11 gene. The gene is localized on chromosome 2, region q31.1 and consists of 28 exons. According to the literature, the most frequent Caucasian-specific single nucleotide polymorphism

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in the ABCB11 gene is 1331T > C (rs2287622). The transition of thymine into cytosine translates into alanine in place of valine at the position 444 of the protein sequence [6, 7].

Another potent phospholipid transporter is involved in bile shuttling, namely a Multidrug Resistance Protein 3 (MDR3). The MDR3 protein is encoded by ABCB4 gene [8, 9]. It is found on chromosome 7, position 7q21.12 [10, 11]. One of the most frequently analyzed ABCB4 gene polymorphism is 1954A > G in exon 16 (rs2230028). The result of transition of adenine into guanine results in substitution of arginine with glycine at the position 652 of protein sequence (R652G, Arg652Gly).

Both BSEP and MRD3 are a part of MDR/TAP protein family composed of ATP-active membrane transporters ABC. ABC proteins transport many different molecules through the cellular membrane and a reduction of its expression may lead to excessive accumulation of toxic metabolites within cells [12–14].

There are some observations suggesting that hereditary impairment of ABCB4 and ABCB11 gene products due to gene polymorphism may be a reason of hepatic cirrhosis in early childhood [15, 16]. Thus, the dysfunctional ABCB4 and ABCB11 genes may also be responsible for the development of ICP.

The aim of the study was to explore the possible role of gene variants encoding ABCB-type transporters in the etiology of ICP. The frequency of single nucleotide polymorphism was analyzed in two genes that encode the export pumps of bile acid salts: ABCB 11 (BESEP) (1331T > C, V444A polymorphism) and ABCB4 (MDR3) (1954A > G, R652G polymorphism).

MATERIAL AND METHODS

A total of 96 women with ICP diagnosed in the second half of pregnancy (mean age 30.42 ± 4.38 years, mean gestational age 36.83 ± 2.75 gw.) and 211 healthy pregnant women (mean age 30.68 ± 4.67 years, mean gestational age 39.05 ± 1.22 gw.) were enrolled into the study. The analysis was performed at the Division of Perinatology and Women's Diseases of Poznan University of Medical Sciences in Poznan and at the Department of Gynecology and Obstetrics with Gynecological Oncology Subdivision of Regional Hospital in Zielona Góra in the years 2013–2017 (Poznan University of Medical Sciences Bioethics Committee permission no 842/13). All subjects were informed about the goal of the study and gave their written consent.

The ICP was diagnosed on the basis of clinical and biochemical criteria such as: typical itching without rush, fasting total bile acids (TBA) serum concentration higher than 10 µmol/L, increased concentration of aminotransferase (ALT, AST), symptoms subside within 2–3 weeks after delivery. The exclusion criteria were as follows: infection with hepatitis virus type A, B or C, autoimmune diseases, excessive alcohol drinking, HIV infection and all kinds of hepatic impairments and dermatological diseases that are connected with itching. All women were Caucasian race, Polish origin with singleton pregnancy.

Table 1. Primer sequences used for PCR analysis

SNP	Primers for PCR amplification	PCR product
ABCB11 1331T > C	5′- ACT TCT Tgg TCA Tgg CTC TCA g -3′ 5′-ACT TgA TCT gCA ATg CCA AC-3′	723 bp
ABCB4 1954A > G	5′- TCC TTg ATT gAg AAg Cag TTA gg-3′ 5′- CAA AgA gTA Tgg CTC ATA gTA gC -3′	457 bp

The following records were taken of all the patients from both groups (ICP and control group): detailed obstetrical history, age, gestational age at the delivery, height, weight prior to and at the end of pregnancy, systolic and diastolic blood pressure, mode of delivery and the newborn condition. All lab tests were performed at the Central Laboratory of Gynecological-Obstetrical Hospital of Poznan University of Medical Sciences.

Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. Genotyping was performed at the Laboratory of Molecular Biology of Poznan University of Medical Sciences using polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) method with primers published by Dias [17] and Bronsky et al. [18] (Tab. 1). The obtained DNA fragments were hydrolyzed with the following restriction enzymes: BsuRI for ABCB11 and TspRI for ABCB4.

The observed results and clinical data of patients and newborns were analyzed statistically with R software (version 3.4.2, http://cran.r-project.org) and $p < 0.05$ was considered statistically significant.

RESULTS

Analysis of clinical and laboratory data

The analysis of both ICP and control groups has revealed some statistically significant differences in the duration of pregnancy (36.83 ± 2.75 gw. in ICP group vs. 39.05 ± 1.22 gw. in controls, $p < 0.0001$). Weight gain throughout pregnancy was lower in the ICP group compared to controls (4.21 ± 1.98 vs. 5.42 ± 1.74, $p < 0.0001$). Also, the ICP group had a statistically significant ($p < 0.0001$) lower body mass of newborns and a slightly lower 5th minute Apgar score compared to the controls (3083.00 ± 634.95 g vs. 3425.00 ± 433.82 and 9.74 ± 0.75 vs. 9.97 ± 0.23 respectively). The interesting observation made was a lower placental mass in the ICP group compared to the controls (579.11 ± 150.93 g vs. 620.18 ± 111.37 g, $p = 0.02$). The other values: first minute Apgar score or arterial and venous pH values were not statistically different between the groups.

Analysis of 1331T > C ABCB11 and 1954A > G ABCB4 polymorphism

The genetic comparison of the ICP and the control group regarding the 1331T > C ABCB11 polymorphism has shown a similar frequency of homozygote 1331TT genotype (11.46% vs. 18.01% respectively, OR = 0.59, p = 0.10) and of heterozygote 1331TC genotype (48.96% vs. 52.61% respectively, OR = 0.86, p = 0.32). A mutated homozygote 1331CC genotype was more frequent in the ICP group when compared to the controls (39.58% vs 29.38%, OR = 1.57, p = 0.05).

The frequency of a wild-type 1331T allele was higher in the control group (35.94% vs. 44.31%, p = 0.03), whereas the frequency of a mutated 1331C allele was higher in the ICP group (64.06% vs. 55.69%, OR = 1.42, p = 0.03) (Tab. 2).

The analysis of 1954A > G ABCB4 polymorphism has shown that the homozygote 1954AA ABCB4 genotype and the heterozygote 1954AG ABCB4 genotype had similar frequencies across the ICP and the control group (respectively: 21.88% vs 17.54%, OR = 1.32, p = 0.23 and 78.12% vs 82.46%, OR = 0.76, p = 0.23). We have not observed the presence of a mutated homozygote 1954GG ABCB4 genotype in neither of the investigated groups. Similar observation applies to alleles' frequency i.e., the presence of both alleles 1954A and 1954G was similar in both investigated groups (for 1954A allele: 89.06 vs. 91.23%, OR = 0.78, p = 0.24, for 1954G allele: 10.94 vs. 8.77%, OR = 1.28, p = 0.24) (Tab. 3).

Analysis of 1331T > C ABCB11 and 1954A > G ABCB4 co-existence

The cross-tabulation of genotype combinations of 1331T > C ABCB11 and 1954A > G ABCB4 is displayed in Table 4. The higher frequency of genotypes containing 2 mutated alleles for 1331T > C ABCB11 polymorphism was shown in ICP group then in controls: 1331CC/1954AA (31.25 vs. 25.12, p = 0.16) and 1331CC/1954AG (8.33 vs. 4.27, p = 0.12).

Table 2. The distribution of genotypes and alleles of ABCB11 gene 1331T > C polymorphism among women with ICP and controls

ABCB11 1331T > C	Study group ICP (n = 96)		Control group (n = 211)		OR	95% CI	p
Genotypes	**Observed value n (%)**	**Expected value [%]**	**Observed value n (%)**	**Expected value [%]**			
TT	11 (11.46)	12.92	38 (18.01)	19.64	0.59	0.29–1.21	0.10
TC	47 (48.96)	46.04	111 (52.61)	49.35	0.86	0.53–1.40	0.32
CC	38 (39.58)	41.04	62 (29.38)	31.01	1.57	0.95–2.61	**0.05**
Total	96 (100.00)	100.00	211 (100.00)	100.00			
Alleles							
T	**69 (35.94)**	–	**187 (44.31)**	–	**0.71**	**0.50–1.00**	**0.03**
C	123 (64.06)	–	235 (55.69)	–	1.42	1.00–2.02	**0.03**
Total	192 (100.00)	–	422 (100.00)	–			

HWE: ICP p = 0.54; CONTROL p = 0.34

Table 3. The distribution of genotypes and alleles of ABCB4 gene 1954A > G polymorphism among women with ICP and controls

ABCB4 1954A > G	Study group ICP (n = 96)		Control group (n = 211)		OR	95%CI	p
Genotypes	**Observed value n (%)**	**Expected value [%]**	**Observed value n (%)**	**Expected value [%]**			
AA	75 (78.12)	79.32	174 (82.46)	83.23	0.76	0.42–1.38	0.23
AG	21 (21.88)	19.48	37 (17.54)	16.00	1.32	0.72–2.40	0.23
GG	0 (0.00)	1.20	0 (0.00)	0.77	–	–	–
Total	96 (100.00)	100.00	211 (100.00)	100.00			
Alleles							
A	171 (89.06)	–	385 (91.23)	–	0.78	0.44–1.38	0.24
G	21 (10.94)	–	37 (8.77)	–	1.28	0.73–2.25	0.24
Total	192 (100.00)	–	422 (100.00)	–			

HWE: ICP p = 0.23; CONTROL p = 0.16

Table 4. The co-existence rate of the 1331T > C and the 1954A > G polymorphisms in the respective ABCB11 and ABCB4 genes of the studied groups

			ABCB4 1954A > G			Total
			AA	AG	GG	
Study group ICP (n = 96)	ABCB11 1331T > C	TT	9 (9.38)	2 (2.08)	0 (0.00)	11 (11.46)
		TC	36 (37.5)	11 (11.46)	0 (0.00)	47 (48.96)
		CC	30 (31.25)	8 (8.33)	0 (0.00)	38 (39.58)
		Total	75 (78.13)	21 (21.88)	0 (0.00)	96 (100.00)
Control group (n = 211)	ABCB11 1331T > C	TT	29 (13.74)	9 (4.27)	0 (0.00)	38 (18.01)
		TC	92 (43.60)	19 (9.00)	0 (0.00)	111 (52.61)
		CC	53 (25.12)	9 (4.27)	0 (0.00)	62 (29.38)
		Total	174 (82.46)	37 (17.54)	0 (0.00)	211 (100.00)

DISCUSSION

1331T > C ABCB11 and 1954A > G ABCB4 polymorphisms

Membrane transporters ABCB4 and ABCB11 are responsible for the removal of the bile acids from hepatocytes. Consequently, their mutated genetic variants may be involved in ICP etiology [9, 19]. The polymorphism that has received most attention is 1331T > C in the ABCB11 gene that encodes BSEP.

The role of 1331T > C polymorphism of the ABCB11 gene has been confirmed in our study. In the group of a total of 96 women with ICP, we have observed a higher frequency of mutated homozygotic genotype 1331CC (39.58% vs 29.38%, OR = 1.57, p = 0.05) and a higher frequency of mutated 1331C allele (64.06% vs. 55.69%, OR = 1.42, p = 0.03) when compared to ICP-free controls.

Other authors have obtained similar results. Meier et al. has shown a probable association between the 1331T > C ABCB11 gene polymorphism and the occurrence of ICP or contraceptive-induced cholestasis (CIC) (4 women with CIC, 41 with ICP, 205 healthy subjects, 40 healthy pregnant women). All women with CIC were carriers of the mutated 1331C allele. The lowest TBA level was observed in patients with the 1331TT genotype and the highest in patients with the 1331CC genotype. The authors have concluded that a strong dysfunction of bile acid transporters BSEP caused by the presence of 1331CC ABCB11 genotype could lead to the development of cholestasis during oral contraception. It has also been revealed that the presence of 1331CT ABCB11 genotype could be a reason of developing ICP [9].

Interestingly, women from Asian populations who are known to have a lower incidence of ICP have also been suggested to develop the pathology in association with ABCB4 and ABCB11 gene polymorphisms. Kamimura et al. reports a first Japanese case of a pregnant women with ICP associated with 1331T > C (rs2287622, V444A) ABCB11 and 504C > T (rs1202283, N168N) ABCB4 gene polymorphisms [20].

Many studies focus on a few polymorphisms at once. Wasmuth et al. has analyzed a few polymorphisms of ABCB4 and ABCB11 genes and its haplotypes in Swedish population (e.g. 1331T > C ABCB11 and 1954A > G ABCB4). In a sample of 45,485 pregnant women, 937 reported itching (2.1%), of which 693 were diagnosed with ICP (TBA ≥ 10 µmol/L) (1.5%) and 130 (0.3%) with a severe ICP (TBA ≥ 40 µmol/L). There were no differences as to the frequency of ABCB11 gene haplotypes. Yet, some variants of ABCB4 gene haplotype have shown to be a risk factor for a severe ICP. The ABCB4_5 haplotype in particular was observed more frequently in the study group (5.8% vs. 0.9%) [21].

In our study we have also analyzed a 1954A > G ABCB4 gene polymorphism in the ICP group. We have found no statistically significant difference in the frequency of genotypes and alleles. Similar observations are reported by Bacq et al. who have investigated 8 polymorphisms of ABCB4 gene that is involved in the MDR3 synthesis (50 women with ICP, 107 controls, Caucasian race). No correlation was found between 1954A > G ABCB4 gene polymorphism and the development of ICP. Yet, a statistically significant difference was revealed as to the frequency of Arg590Gln ABCB4 gene polymorphism, which was higher in the ICP group compared to the controls (p = 0.0017, OR = 16.03) [22].

Coexistence of 1331T > C ABCB11 and 1954A > G ABCB4 gene polymorphism

Our study has also performed the analysis of coexistence of polymorphisms in ABCB4 and ABCB11 genes. Higher frequency of coexistence of genotypes containing 2 mutated alleles of 1331T > C ABCB11 polymorphism and 1954A > G variants of ABCB4 gene in ICP group was revealed. These observations are confirmed by other studies. For instance, Dixon et al. have investigated 6 genes involved in the etiology of ICP by analyzing 83 polymorphisms in ABCB4, ABCB11, ABCC2, ATP8B1, NR1H4 and FGF19 gene. The ICP group consisted of 563 patients whereas the control group

consisted of 642 healthy pregnant women, all of Caucasian race. The study revealed that the key role in ICP etiology is played by the coexistence of polymorphisms of ABCB4 and ABCB11 genes [19, 23].

Anzivimo et al. arrive to similar conclusions (33 pregnant patients with ICP, Italian population). The authors have revealed 5 new mutations (2 in ABCB4 gene: 1587DfsX603, 1738LfsX744 and 3 in ABCB11 gene: V284D, Q558H, P731S). The most severe ICP was observed in carriers of I587DfsX603, I738LfsX744 and V284D mutations [24].

Such strong correlation of various dysfunctional genetic variants involved in the bile acid metabolism confirms the hypothesis that the impairment of bile transport present in ICP can be caused by a dysfunctional genetic variants, especially when in combination. In the course of ICP sudden intrauterine fetal demise may be observed. In turn, in severe ICP the frequency of intrauterine fetal demise is as high as 5.6% [21, 25–28].

CONCLUSIONS

Overrepresentation of mutated genetic variants of 1331T > C ABCB11 in the ICP group suggests its role in the ICP etiology. The analysis of coexistence of the investigated polymorphisms shows the influence of 1954A > G ABCB4 and 1331T > C ABCB11 on the risk of ICP development. Yet, there is a need to continue genetic studies on larger groups of patients.

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