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The influence of common gene variants of the xenobiotic receptor *(PXR)* in genetic susceptibility to intrahepatic cholestasis of pregnancy

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SUMMARY

Background
The xenobiotic nuclear pregnane X receptor is implicated in many physiological pathways and diseases, including bile acid detoxification and cholestasis.

Aim
To estimate the contribution of common gene variants of the xenobiotic receptor (pregnane X receptor, *PXR)* to genetic susceptibility to intrahepatic cholestasis of pregnancy.

Methods
A total of 101 intrahepatic cholestasis of pregnancy patients and 171 healthy pregnant women in the third trimester of their pregnancies were included. Four tag single nucleotide polymorphisms (SNPs) (rs12488820 C/T, rs2472671 C/T, rs2461823 A/G, and rs1054191 A/G) encompassing 36 kb in chromosome 3, with a minor allele frequency ≥0.10 and representing 33 polymorphic sites were genotyped. Besides these, three additional SNPs (rs3814057, rs6785049, and rs7643645) were included because they showed previous evidence of functionality.

Results
Genotypic test for single SNPs showed that rs2461823 genotypes were significantly associated with intrahepatic cholestasis of pregnancy ($P < 0.0069$), OR per G allele: 1.44, 95% CI: 1.01–2.05, $P < 0.042$. The Cochran-Armitage test for trend and the allelic test showed a significant association with disease status ($P < 0.04$ and 0.03 respectively), G being the risk allele. A positive association between rs2461823 and ALT, AST, and bilirubin concentrations was observed. Neonate birth weight adjusted by the Capurro index was significantly associated with rs2461823 ($P < 0.05$); the proportion of the total variation attributed to rs2461823 genotypes was 7.8%.

Conclusion
Common *PXR* polymorphisms may contribute to the genetic susceptibility to intrahepatic cholestasis of pregnancy.

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INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a liver disorder characterized by pruritus and raised serum bile acid levels, most often occurring during the third trimester of pregnancy.

Intrahepatic cholestasis of pregnancy poses little maternal risk; however, it can lead to prematurity, foetal distress and intrauterine death.[1, 2]

While the pathogenesis of ICP remains unknown, several lines of evidence show that the aetiology of the disease is likely to involve environmental, hormonal and genetic factors.[3, 4] For instance, the disease has a particular geographical distribution, its prevalence being higher in Chile, Bolivia, Scandinavia and China compared with any other population.[3]

Such is the importance of the ethnic predisposition to develop intrahepatic cholestasis of pregnancy that the prevalence of the disease in Chile, for example, ranged in 1978 from 13.8% in women of Araucanian Indian descent to 3.9% in women of Aimara Indian descent;[5] a similar picture was reported about the ICP prevalence in Bolivia.[6] A detailed explanation of the reported incidence of ICP in different countries and ethnic groups was recently published.[7]

The seasonal variation of ICP in countries with high prevalence also suggests the involvement of environmental factors in its aetiology.[8] It was described that the incidence of ICP peaks in the winter months in Chile, an observation that coincides with the apparently lower plasma levels of selenium in winter.[4]

Moreover, selenium deficiency[9] or excess of long-chain monounsaturated fatty and erucic acid[10] was postulated as playing a role in ICP. A recent report further included the possibility of a 'leaky gut' as an additional environmental factor that may participate in the pathogenesis of ICP by enhancing the absorption of bacterial endotoxin and the enterohepatic circulation of cholestatic metabolites of sex hormones and bile salts.[11]

The influence of hormonal factors, in particular oestrogens and progesterone, in the aetiology of ICP is strongly supported by previous studies, as comprehensively reviewed by Arrese and Reyes.[8, 12]

Finally, familial clustering strongly indicates a genetic predisposition for ICP. ICP was reported in sisters and mothers of ICP patients[13–15] and the observation that ICP affects a large degree of kindred raised the hypothesis that the disease may be transmitted as a predisposing trait.[3] Additional insights into the genetic aetiology of ICP are given by the many candidates genes that were explored as responsible for genetic susceptibility to the disease, as thoroughly reviewed recently by Dixon and Williamson.[16] Interestingly, most of the reports about genetic loci associated with ICP are mainly focused on hepatobiliary transporters and either rare mutations or common gene variants were described, suggesting that ICP is a complex disease. In addition, functional variants in the farnesoid X receptor (*FXR*) were also implicated in the genetic predisposition to ICP in a European population, suggesting a putative role of the genes controlling bile acid homeostasis.[17, 18]

Pregnane X receptor (*PXR)* – also known as nuclear receptor subfamily 1, group I, member 2 (*NR1I2*), steroid and xenobiotic receptor (*SXR*), or pregnane-activated receptor (*PAR*) – is a member of the nuclear receptor superfamily, whose primary function is the regulation of an entire network of genes involved in the detoxification and elimination of xenobiotics from the body, including their oxidation, conjugation and transport.[19] In addition, *PXR* coordinately regulates several genes involved in bile acid metabolism.[19] Genetic polymorphisms in the *PXR* may explain the interindividual variation in the induction of drug transporters and physiological or pathophysiological changes in bile acid levels.[20]

Given the complex aetiology of ICP, in which several endogenous and exogenous factors interact in genetically susceptible women to express the disease, we focused on the selection of a candidate gene, such as *PXR*, which may be implicated not only in xeno- and endobiotic response but also in bilirubin and bile acid detoxification, as well as ABC-transporter gene induction. Hence, we speculate that common *PXR* gene variants may contribute to differences in individual susceptibility to ICP. Additionally, we explored the relationship between *PXR* gene variants and the obstetrical and perinatal characteristics of the newborns of ICP mothers.

PATIENTS AND METHODS

Settings and study design

Between 1 June 2003 and 1 December 2007, we performed a cross-sectional study in normal pregnant women and patients with ICP in a county hospital of the city of Buenos Aires. ICP incidence was 1.1% (data were calculated over 2300 pregnancies/year on average).

Patients

A total of 101 ICP patients at the Department of Obstetrics and Gynecology who were referred to the Liver Section and 171 unrelated healthy pregnant women in their third trimester of pregnancy who visited the hospital during the study period were included.

All the patients with intrahepatic cholestasis of pregnancy admitted to the Liver Section were born in Peru, Bolivia and Paraguay. Thus, the control group was selected in accordance with this geographical and ethnic parameter and the same proportion of women in both cases and controls in the three neighbouring countries of Argentina were included, and to ensure homogeneity of the genetic background, the control population was matched with affected individuals on ethnicity, area of residence and time of recruitment.

Diagnosis of ICP was based on the following criteria: (1) the presence of pruritus occurring during the second half of an otherwise uneventful pregnancy; (2) the presence of abnormalities in the liver function test suggestive of ICP: serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) greater than 40 or 35 IU/L respectively; (3) elevated levels of fasting total bile salts (SBA) >12 μmol/L; (4) no skin lesions caused by systemic diseases that could cause pruritus; and (5) spontaneous resolution of clinical symptoms and laboratory findings after delivery. All the patients were referred for evaluation during pregnancy. Additional inclusion criteria were the absence of infection by hepatitis viruses (HAV, HBV, and HCV; AxSYM, Abbott, Buenos Aires, Argentina) autoimmune diseases, moderate-to-severe alcohol intake, HIV infection (Enzyme-Linked Immunoabsorbent Assay, Organon Teknika, Buenos Aires, Argentina), biliary obstruction and the use of drugs or alternative medicine therapy known to precipitate cholestasis.

Patients with a verified family history of ICP were classified as familial ICP, while those without a family history (or whose family history could not be verified) were termed sporadic cases.

Complete medical, obstetrical and perinatal data were recorded. Apgar score[21] and gestational age assessed by the Capurro method were registered in all newborns.[22]

In addition, all relevant clinical variables, such as gestational age, birth weight and perinatal morbidity were registered for each pregnancy.

METHODS

Liver function tests

Serum samples were obtained after a fasting period of 12 h, and aliquots were frozen at −20 °C until bile acid determinations were performed. Serum alanine (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamil-transpeptidase (GGT) and total and conjugated bilirubin concentrations were carried out by routine automated techniques.

Serum total bile acids were measured by capillary electrophoresis as previously reported (normal value <12 μmol/L).[23]

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board and the Bioethical Committee of our institution. Written consent was obtained in every case.

Genotype and Haplotype analysis

To assess the contribution of *PXR* gene variants to ICP, we selected tag single nucleotide polymorphisms (tagSNPs) using the Tagger computer program.[24] A tagSNP is a representative SNP of other untyped SNPs in a region of the genome with high linkage disequilibrium (LD) that allows identifying genetic variation without genotyping every SNP in a chromosomal region.

We used the aggressive tagging method (performed by aggressively searching for multimarker predictors as effective surrogates for single tag SNPs) to construct a single marker or multimarkers to capture alleles of interest based on the computed correlation r^2 between them, and the phase II genotyping data from the HapMap project for Caucasians from the CEU dataset (Utah residents with ancestry from northern and western Europe), with a minor allele frequency (MAF) ≥0.10 and a minimum correlation coefficient at which all alleles are to be captured (r^2) of 0.8.[24]

In addition, we included three more SNPs (rs3814057-a SNP located in the 3′ un-translated gene region, rs6785049-an intronic SNP, and rs7643645-a SNP located in the gene promoter region) because previous reports showed evidence of them being functionally associated with altered regulation of critical downstream effector genes involved in xenobiotic removal (*CYP3A4* and *MDR1*).[25, 26]

Genotyping of the *PXR* gene variants was performed by a high-throughput genotyping method (Prevention

Genetics, Marsfield, WI, USA) involving PCR amplification of genomic DNA with two-tailed allele-specific primers that introduce priming sites for universal energy-transfer-labelled primers, as previously described.[27]

PLINK software was used for assessing the association between SNPs, and affection status and quantitative traits, and for testing for Hardy–Weinberg equilibrium.[28]

We tested crude associations of each polymorphism with the disease trait under the assumption of an additive model of inheritance. When appropriate, additional dominant and recessive genetic models were assessed after visual inspection of the results to gain a better predictive model.

To ensure genotyping quality, we included DNA samples as internal controls, hidden samples of known genotype, and negative controls (water). Genotypes with a signal below a negative control were not scored.

Statistical analysis

Quantitative data are expressed as median and range unless otherwise indicated. We assessed the differences between groups by Mann–Whitney *U*-test, Kruskall–Wallis test, or two-way ANOVA after log transformation of the variables. In some cases, the association of disease-related quantitative traits with genotypes was tested by linear regression analysis.

The association of disease status and genotype frequencies was analysed by logistic regression and chi-square, d.f. = 2. Differences in allele frequencies for each SNP, and family history of disease between case subjects and control subjects were tested with a chi-square test with 1 degree of freedom. Cumulative odds ratios and 95% confidence intervals using a proportional odds model (Liu-Agresti method[29]) were estimated, and *P* stands for nominal *P* for the extended Mantel-Haenszel test for trend.

Population attributable risk (PAR) was estimated for SNPs that remained significant after adjustment for other covariates with the use of the following equation: PAR% = 100 × *P* (odds ratio−1) / [*P* (odds ratio−1) + 1], where *P* is the probability of a control having the risk genotype, and PAR% is the PAR percentage.[30]

We used the CSS/Statistica program package STATSOFT V 6.0 (Tulsa, OK, USA) to perform these analyses.

RESULTS

The clinical, obstetrical and perinatal characteristics of the ICP patients and healthy pregnant women are shown in Table 1. Family history of ICP was significantly different in cases and controls ($P < 0.0001$). Among the ICP patients, 22 mentioned a family history of the disease, while 57 cases were sporadic. The remaining 22 patients did not know of any family history of the disease. In the control group, no woman

Table 1. Clinical, biochemical, obstetrical and perinatal characteristics of patients with intrahepatic cholestatis of pregnancy (ICP) and healthy pregnant women

Characteristics	ICP patients Median, IQR	Healthy pregnant controls Median, IQR	*P* level
Age (years)	27, 10.0	25, 9.0	N.S.
Number of pregnancies	2, 2.0	2, 2.0	N.S.
Gestational age at delivery (weeks)	36, 5.0	38,5.5	<0.001
Neonate birth weight (gr.)	3050, 600	3400, 700	<0.001
Neonate Apgar score	9, 0	10, 1	<0.001
Total bilirubin (μmol/L)	12, 9	6, 4	<0.001
Direct bilirubin (μmol/L)	3, 4	1, 1	<0.001
Asparte aminotransferase (IU/L) AST	45, 40	17, 7	<0.001
Alanine amino transferase (IU/L) ALT	47, 78	14, 8	<0.001
Alkaline phophatase (IU/L)	620, 365	232, 148	<0.001
Gamma glutamil-transpeptidase (IU-L)	19, 87	18.5, 20	N.S.
Serum total bile acids (μmol/L)	25.83, 23.75	10, 9.6	<0.0001

Results are expressed as median, range. *P* level indicates statistical significance using Mann–Whitney test. IQR, Interquartile range.

showed a family history of ICP. In both cases and controls, women had singleton deliveries.

Gestational age at delivery, birth weight, and Apgar score for neonates of ICP patients were significantly lower compared with those of controls (Table 1). As expected, liver function tests in patients with ICP significantly differed from those in healthy pregnant women (Table 1).

PXR gene variants

The human *PXR* gene is located at 3q12-q13.3 and consists of 9 exons; exons 2–9 contain the coding region for a 434 amino acid protein.[26]

To minimize the number of markers selected for genotyping (the HapMap B35 full set database includes 62 polymorphic sites with a MAF>0.05), we selected 4 tag SNPs showing a minor allele frequency ≥10% (rs12488820 C/T, rs2472671 C/T, rs2461823 A/G, and rs1054191 A/G), encompassing 36 kb in chr.3 (120984247 to 121020021), and representing 33 polymorphic sites ($r^2 \geq 0.8$) considering the HapMap project data.

Genotyping success rate was 95.9% for rs12488820, 94.2% for rs2472671, 97.1% for rs2461823, 93% for rs7643645, 99.3% for rs6785049, 94.2% for rs1054191 and 95.6% for rs3814057.

No marker showed departure from Hardy–Weinberg equilibrium, indicating robust genotyping performance in this study (Table 2).

In univariate analysis, the comparison of the genotype frequencies of the six SNPs in cases and controls showed that rs2461823 was significantly associated with the disease (nominal $P < 0.0069$ using chi-square, d.f. = 2), genotypic OR per G allele: 1.44, 95% CI: 1.01–2.05, $P < 0.042$. This significant association remained even when the most conservative Bonferroni correction was applied ($P < 0.042$). The genotype dis-

Table 2. Genotype distribution of seven SNPs in cases and controls

dsSNP rs*	Genotype	ICP patients *n*	Controls *n*	Cumulative OR (95% CI)	*P* value
rs12488820	CC	32	67	1.27 (0.79–2.02)	N.S.
	CT	52	68		
	TT	16	27		
rs2472671	CC	1	2	0.80 (0.41–1.53)	N.S.
	CT	19	26		
	TT	74	134		
rs2461823	AA	20	61	1.65 (1.024–2.64)	<0.041
	AG	59	68		
	GG	22	34		
rs7643645	AA	18	31	0.75 (0.4601.21)	N.S.
	AG	47	72		
	GG	24	60		
rs6785049	AA	48	75	0.77 (0.43–1.25)	N.S.
	AG	44	77		
	GG	7	19		
rs1054191	AA	1	1	0.80 (0.43–1.50)	N.S.
	AG	20	32		
	GG	69	133		
rs3814057	AA	58	112	1.13 (0.70–1.95)	N.S.
	AC	33	49		
	CC	2	6		

* dsSNP ID: Single Nucleotide Polymorphisms on NCBI Reference Assembly. Odds ratios (OR) and 95% confidence intervals (95%CI). Cumulative OR using proportional odds model is shown. Cumulative OR stands for the cumulative effect of the two genotypes (heterozygous and homozygous in comparison with homozygous for the other allele in the first row, which was taken as the reference group). *P* value stands for nominal *P* for the extended Mantel-Haenszel test for trend.

tribution of the seven SNPs in cases and controls is shown in Table 2. The allelic association test (OR for the G allele: 1.45, 95% CI: 1.02–2.07) and Cochran-Armitage test for trend for rs2461823 were also significantly associated with ICP ($P < 0.03$ and 0.04 respectively). In addition, there was a more significant association with the disease under the dominant model, GG+AG vs. AA rs2461823genotypes, (OR: 2.42, 95% CI: 1.35–4.35, $P < 0.003$). The population attributable risk percentage (PAR%) for the risk genotype (rs2461823 GG) was 15.25% (95% confidence interval: 1.79–30.03).

Disease-associated quantitative traits were additionally tested for association with the *PXR* SNPs. Serum levels of ALT and AST were significantly associated with rs2461823 (beta ± SE: 18.2 ± 7.5, $P < 0.01$ and beta ± SE: 12.0 ± 5.0, $P < 0.01$ respectively). A positive association with rs2461823 was also observed for total and conjugated bilirubin concentrations (beta ± SE: 0.08 ± 0.03, $P < 0.01$ and beta ± SE: 0.03 ± 0.018, $P < 0.03$ respectively) (Figure 1). In addition, SBA concentrations were significantly higher in the rs2461823-GG genotype (mean ± SE: 10.3 ± 3.0 μmol/L) and GA genotype subjects (mean ± SE: 12.9 ± 2.1 μmol/L) vs. the AA genotype subjects (6.7 ± 2.6 μmol/L), $P < 0.05$, Kruskal–Wallis test. No association was observed with the multimarkers and the disease.

Neonate birth weight adjusted by the Capurro index was significantly associated with rs2461823 (Mean ± S.E.; GG: 2908 ± 85, AG: 2985 ± 54, and AA: 3204 ± 87; $P < 0.05$); the proportion of the total variation attributed to the rs2461823 genotypes was 7.8%. The Apgar score was significantly correlated with rs2461823 (Spearman R: 0.19, $P < 0.02$). No significant association was observed with any obstetrical and perinatal characteristics.

None of the other evaluated SNPs showed significant associations either with ICP or other disease-associated traits.

The functional characteristics of the SNPs tagged by rs2461823 are shown in Table 3. As it is shown, the rs2461823 is a proxy of 4 other *PXR*-variants located in the gene regulatory region, of which rs2472677 (in very strong LD with the rs2461823, r^2: 0.93), for instance, is a SNP with previous knowledge about functionality as a modulator of the activity of the CYP3A4 (a member of the cytochrome P450 superfamily of enzymes that catalyse many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids).

DISCUSSION

We performed a candidate-gene association study in a hospital-based population sample of 271 South American women to evaluate the relationship between ICP, and common and previously known functional variants in the *PXR*. Additionally, we explored a possible role of the gene variants on the foetal outcome of the newborns of ICP mothers. We observed that the rs2461823 G allele was significantly associated with ICP and also with several disease-associated traits, suggesting a putative role of *PXR* variants in individual susceptibility to the disease. The rs2461823 G allele adds a 44% higher risk of having the disease; thus, the estimated risk of ICP for women carrying the G allele is 2.5-fold higher in comparison with those carrying AA homozygous alleles. While we did not observe any association with the functional SNPs that we genotyped in our sample, it is worth mentioning that rs2461823 is a tagSNP and represents by itself another linked variant. Interestingly, this SNP is an excellent proxy (r^2 about 1) of six additional variants (four of them located in the *PXR* promoter region). As previously shown, most of the observed variability in the hepatic expression of *PXR* and its targets genes (*CYP3A4* and *MDR1*) may be explained by genetic variants located in the promoter regulatory region.[25] As shown in Table 3, some of the tagged SNPs create or destroy putative transcription factor binding sites such as HNF3β, which belongs to a transcriptional network that regulates the expression of hepatocyte-specific genes required for bile acid biology.[31]

Nevertheless, it is worth noting that given the extent of LD in the human genome, it is difficult to identify the causal variant, and because of that, the casual variant could be another common variant, a rare variant, or a structural variant.[32] Therefore, any effort to evaluate the potential functionality of the associated tagSNP, either in *in vitro* or in *in vivo* models, may result impractical and somehow of limited concordance with the epidemiological evidence.[32]

Some limitations about the genetic approach of evaluating common variants (as said before SNPs with a minor allele frequency higher than 10%) should be mentioned. In particular, because it is likely that rare variants in the *PXR* gene also play a role in ICP.

Consistent with the relation between rs2461823-G and ICP, some disease-associated traits were significantly associated with the variant; total bilirubin lev-

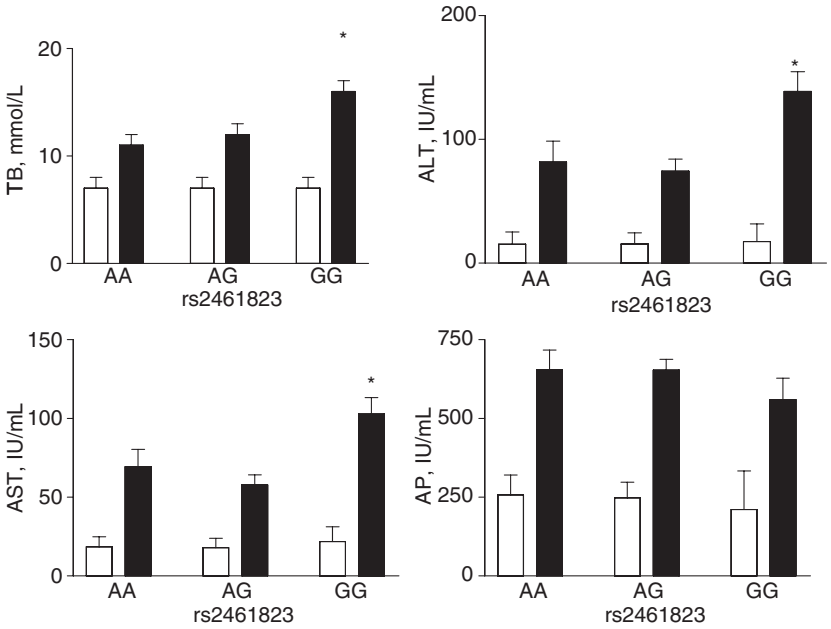

Figure 1. Bar plots of disease-associated traits in ICP cases and controls according to the *PXR* rs2461823 genotypes. ICP, intrahepatic cholestasis of pregnancy; TB, total bilirubin (μmol/L); ALT and AST, serum alanine and aspartate aminotransferase; AP, alkaline phosphatase. Results are expressed as mean ± S.E. *$P < 0.01$. P value stands for statistical significance using two-ways ANOVA. White bars represent control pregnant women, and black bars represent ICP cases. In all variables, ICP cases showed significantly ($P < 0.001$) higher levels than healthy pregnant women.

Table 3. Functional characteristics of the markers of *PXR* in strong linkage disequilibrium with the SNP significantly associated with intrahepatic cholestasis of pregnancy: rs2461823

Position in AF364606*	Position from translation start site	rs number NCBI†	Functional effects
63396C>T	−6994	rs2472677	‡The CC homozygote of rs2472677 is associated with a high level of *NR1I2* (*PXR*) gene induction by rifampicin as measured by increased activity of CYP3A4. Samples with the T allele had higher basal levels of NR1I2 mRNA and higher basal activity of CYP3A4. This SNP is predicted to be within a FOXA2 (HNF3beta) transcription factor binding site[25]
63877T>C	−6513	rs6438546	(CAAA)CA insertion
68943C>A	−1447	rs2461817	SNP present in DR3 and C/EBPγ sites. CREB site lost in T allele
66034T>C	−4356	rs13059232	–

* AF364606: chr3:120968000:121009019.
† Single Nucleotide Polymorphisms on NCBI Reference Assembly.
‡ Information retrieved from the publicly available Internet research tool PharmGKB (http://www.pharmgkb.org/).

els, and ALT and AST values were significantly higher in women carrying the G allele.

When we analysed the perinatal characteristics of the newborns according to the *PXR* SNPs, we observed that rs2461823 was significantly associated with a low birth weight and Apgar score, even after adjusting for the Capurro index, suggesting that the variant may also be implicated in some foetal complications, either indirectly by modulating susceptibility to ICP in their mothers, or directly by modulating the expression of the *PXR* and the *PXR* network of enzymes that catalyses drugs and xenobiotics.

Adverse perinatal outcomes in pregnancies complicated by ICP are associated with increased foetal morbidity and mortality. Although foetal complications were regarded as irrespective of maternal bile acid levels, some other factors such as uteroplacental insufficiency were postulated as associated with the disease.[33]

Interestingly, previous experimental data showed that lipopolysaccharides downregulate placental *PXR*, *CYP3A11*, and *MDR1A* mRNA expressions.[34] Consequently, one may speculate that the 'leaky gut' above-mentioned as a putative environmental predisposing factor[11] may interact with an altered function of the *PXR*, influencing the susceptibility to develop ICP and foetal complications. Certainly, the mechanisms by which gene variants in the *PXR* participate in foetal complications in ICP deserve further investigation.

There is growing evidence that ICP is characterized by impaired bile acid homeostasis in the mother. Bile acids (cholic acid, deoxycholic acid, and lithocholic acid) activate the nuclear receptors FXR and PXR. Interestingly, only three previous studies on genetic susceptibility to ICP focused on these two candidate genes. Genetic variation in *FXR* was explored by direct sequencing of the coding regions and intron/exon boundaries in 92 British ICP cases of mixed ethnicity, and four novel heterozygous *FXR* variants were reported to be associated with ICP.[17] In addition, the genotyping of two SNPs in the *FXR* (-20647T>G and IVS7-31A>T) in familiar clustering showed an association with an aggravated cholestatic phenotype.[18] Similarly, sequencing and functional assessment of the *PXR* coding region were also performed in 121 Caucasian ICP patients, and a lack of contribution of the coding genetic variation in *PXR* was reported.[35] In this study, however, no regulatory region, such as the promoter, was explored. It is noteworthy to mention that our study is the first to provide evidence for association between common gene variants in the *PXR* (most of them proxies of SNPs of the regulatory region) and ICP. More studies are necessary to confirm and investigate whether our findings in South American patients may be extended to other geographical populations.

Although the precise molecular events related to the contribution of *PXR* gene variants to ICP remain unclear, several lines of evidence support this association. For instance, *PXR* is particularly involved in the detoxification of cholestatic bile acids, and the gene expression regulation involved in the biosynthesis transport and metabolism of bile acids.[19]

In our population, we have previously observed a clear difference in the bile acid profiles between ICP and normal pregnancies.[23] This difference involved a shift towards a hydrophobic composition with higher levels of lithocholic acid (LCA) and free bile acids in ICP. Interestingly, LCA seems to induce its clearance by activating nuclear receptors, in particular, the constitutive androstane receptor (CAR) and PXR.[36] Moreover, activation of *PXR* by LCA protects against the severe liver damage caused by this bile acid by a coordinated regulation of the expression of several genes involved in its detoxification.[19] As a result, *PXR* variants may modulate susceptibility to ICP by altering LCA detoxification.

As a final point, the genetic architecture of ICP is very complex, and its pathogenesis has been related either to a defect in transport proteins disabling the biliary excretion of physiologically occurring metabolites in pregnancy[37] or to a defect in the homeostatic regulation of bile metabolism.[16] ICP is just an example of the diversity of complex traits, not only in their environmental determinants but also in the genetic components of risk. Hence, we cannot rule out gene interactions among the previously mentioned systems, as it was reported that a disrupted bile acid homeostasis leads to interaction among nuclear receptors, transporters, and cytochrome P450.[38] Certainly, more experiments are needed to decipher this complex and relevant clinical problem and to reveal new therapeutic targets. Nevertheless, it is tempting to postulate that PXR may be one of them.

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