Clinics and Research in Hepatology and Gastroenterology (2016) 40, 141—153

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

MINI REVIEW

The pathophysiology of intrahepatic cholestasis of pregnancy

Peter H. Dixon, Catherine Williamson*

Division of Women's Health, 2.30W Hodgkin Building, King's College London, Guy's Campus, SE1 1UL London, United Kingdom

Available online 25 January 2016

Summary A number of liver disorders are specific to pregnancy. Amongst these, intrahepatic cholestasis of pregnancy (ICP), also known as obstetric cholestasis (OC), is the commonest, affecting approximately 1 in 140 UK pregnancies. Patients commonly present in the third trimester with severe pruritus and deranged serum liver tests; bile acids are elevated, in severe cases >40 μmol/L. Although the disease is considered relatively benign for the mother, increased rates of adverse fetal outcomes, including stillbirth, are associated with ICP. As our knowledge of the mechanisms underlying bile acid homeostasis has advanced in the last 15 years our understanding of ICP has grown, in particular with respect to genetic influences on susceptibility to the disease, the role of reproductive hormones and their metabolites and the possible identity of the pruritic agents. In this review, we will describe recent advances in the understanding of this condition with a particular emphasis on how aspects of genetic and reproductive hormone involvement in pathophysiology have been elucidated. We also review recent developments regarding our knowledge of placental and fetal pathophysiology and the long-term health consequences for the mother and child.

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a liver disease specific to pregnancy, affecting approximately 1 in 140 UK pregnancies with a varied global incidence, both geographically and with ethnicity. Historically the most common rates were reported in Chile, particularly in women of native Indian descent [1]. Recently however rates have been reported as much lower, between 1.5—4% [2]. In Europe, ICP has been reported to occur more commonly in winter months in some countries. Further details of the epidemiology of ICP are reviewed in [3].

Affected women usually present in the third trimester of pregnancy with pruritus, commonly localised to the palms of the hands and the soles of the feet. Some women may present much earlier however, occasionally as soon as eight weeks gestation. There is no rash associated with ICP, but affected women can have dermatitis artefacta secondary to scratching. Jaundice is rarely present. Biochemical

* Corresponding author.
E-mail address: catherine.williamson@kcl.ac.uk (C. Williamson).

http://dx.doi.org/10.1016/j.clinre.2015.12.008
2210-7401/

abnormalities associated with the condition are deranged serum liver tests and raised serum bile acids although the extent of these abnormalities is extremely variable. Serum bilirubin is raised in a small proportion of cases. Serum bile acids are increasingly recognised as the most definitive laboratory test for diagnosis [4–7].

Increasingly ICP is recognised to be associated with an abnormal metabolic profile. In addition to the hepatic features there is an increased prevalence of dyslipidaemia and impaired glucose tolerance [8,9], and maternal comorbidity, e.g. gestational diabetes and pre-eclampsia, occurs more commonly in women with ICP [10,11]. The disease usually resolves soon after delivery, and if the biochemical and clinical abnormalities do not return to normal by 4–6 weeks postpartum, investigations should be performed to exclude alternative underlying liver pathology. The severity of pruritus can be extremely distressing.

ICP is associated with increased rates of adverse pregnancy outcome, including spontaneous preterm labour, fetal distress, fetal asphyxial events and intrauterine death [12,13]. A number of studies have demonstrated an association between higher maternal serum bile acid levels and increased rates of fetal complications, in particular when serum bile acids are raised above 40 μm/L [14,15]. In ICP maternal bile acids cross the placenta and accumulate, resulting in a reversal of the trans-placental gradient of bile acid concentrations [16].

Ursodeoxycholic acid (UDCA) is the most commonly used treatment for ICP. This drug is a naturally-occurring tertiary bile acid, normally comprising about 3% of the human bile acid pool. Small studies show evidence of maternal benefit (confirmed by a recent meta-analysis [17]) but no study has been powered to confirm a feto-protective effect of UDCA treatment. However the same meta-analysis was strongly suggestive of a benefit to fetal outcomes, although the number of cases in whom there was a comparison of UDCA and placebo was relatively small. In some cases rifampicin is used as a second-line treatment [18]. For a more comprehensive review of ICP treatment see [5,19].

Although ICP resolves shortly after delivery, accumulating evidence points to lifelong consequences for the mother and child. Population-based analysis of a large cohort of ICP cases identified a substantial increase in risk for hepatobiliary disease later in life [20] in addition to enhanced susceptibility to hepatobiliary cancer, immune disease and cardiovascular disease [21].

Beyond the adverse fetal outcomes, an impact of ICP on the metabolic health of adolescent offspring has been shown, indicating a programming effect of the *in utero* exposure to high bile acids [22].

Bile formation and enterohepatic circulation

Bile acids are synthesized in the liver and are the primary endpoint of cholesterol catabolism. A multi-step enzymatic pathway, involving at least 17 enzymes, accomplishes this. Synthesis is in part governed by the initial rate-limiting step, the 7-alpha hydroxylation of cholesterol, by the cytochrome P450 enzyme CYP7A1. Subsequent to synthesis, the primary bile acids, cholic acid and chenodeoxycholic acid are conjugated with glycine or taurine, making them impervious to cell membranes. The process of bile formation is an energy-driven process, characterised by export of molecules from hepatocytes into the biliary tree against a steep concentration gradient. Bile acids are effluxed into the bile canaliculi by a specific ATP-dependent transporter, the bile salt export pump (BSEP, systematic name ABCB11). In addition to the bile acids themselves, a number of biliary transport proteins are responsible for export of other molecules into bile (Fig. 1). The second major component of bile, phosphatidyl choline (PC), is flopped into the biliary tree by the ATP-coupled transporter MDR3, also known as ABCB4. PC has a vital protective role in the intraluminal space. During bile formation bile acids exported by BSEP form mixed micelles with PC. These complexes serve to protect the luminal epithelium from the toxic and detergent effects of bile salts and hence allow their secretion without damage to the surrounding cells. PC secretion, concurrent with bile salts, is hence essential to maintain adequate bile flow [23].

Aside from these two major components, bile also contains other organic ion conjugates, exported from the hepatocytes via another ATP-driven transporter, MRP2 (ABCC2). These include bilirubin, drug conjugates and other organic ions. A heterodimeric protein complex of two membrane proteins, ABCG5 and ABCG8, also exports cholesterol. MDR1 (ABCB1), a close homologue of ABCB4 but with much broader substrate specificity, exports other drug conjugates. Phosphatidyl serine is flipped from the outer to the inner membrane by another transporter, ATB8B1 (also known as FIC1) (Fig. 1).

Bile is stored in the gall bladder until release, driven by the action of post-prandial cholecystokinin. In the small intestine, bile acids emulsify dietary fats, lipids and fat-soluble vitamins. Bacteria in the gut are responsible for a range of modifications, including de-conjugation and dehydroxylation, resulting in the formation of secondary bile acids, namely deoxycholic acid and lithocholic acid.

Re-absorption via enterocytes is accomplished by specific transporter systems (Fig. 1), and subsequently bile salts return to the liver via the hepatic portal vein. On the hepatocyte sinusoidal membrane, the sodium-dependent taurocholate co-transporter peptide (NTCP) is responsible for the majority of uptake, with the remainder (>20%) mediated by organic anion co-transporting polypeptides (OATPs, also known as SLCOs). This process of active recycling and circulation is extremely efficient. The liver takes up approximately 95% of bile acids with the remaining 5% lost in faeces. This loss is replaced by *de novo* synthesis as described above.

In addition to the dietary emulsification role described above, bile acids are increasingly recognised as key metabolic signalling molecules, acting via a number of receptor-controlled pathways that impact cholesterol, lipid and carbohydrate homeostasis as well as the immune system.

Bile acids are extremely cytotoxic at low concentrations and hence their synthesis and transport is highly regulated by homeostatic mechanisms. In hepatocytes (and enterocytes) the nuclear receptor FXR (farnesoid X receptor) functions as the principal sensor of intracellular bile acid levels. Primary bile acids bind FXR and following heterodimerization with RXR (retinoid-X receptor) the receptor complex translocates to the nucleus and binds to response elements in the promoters of target genes. These target

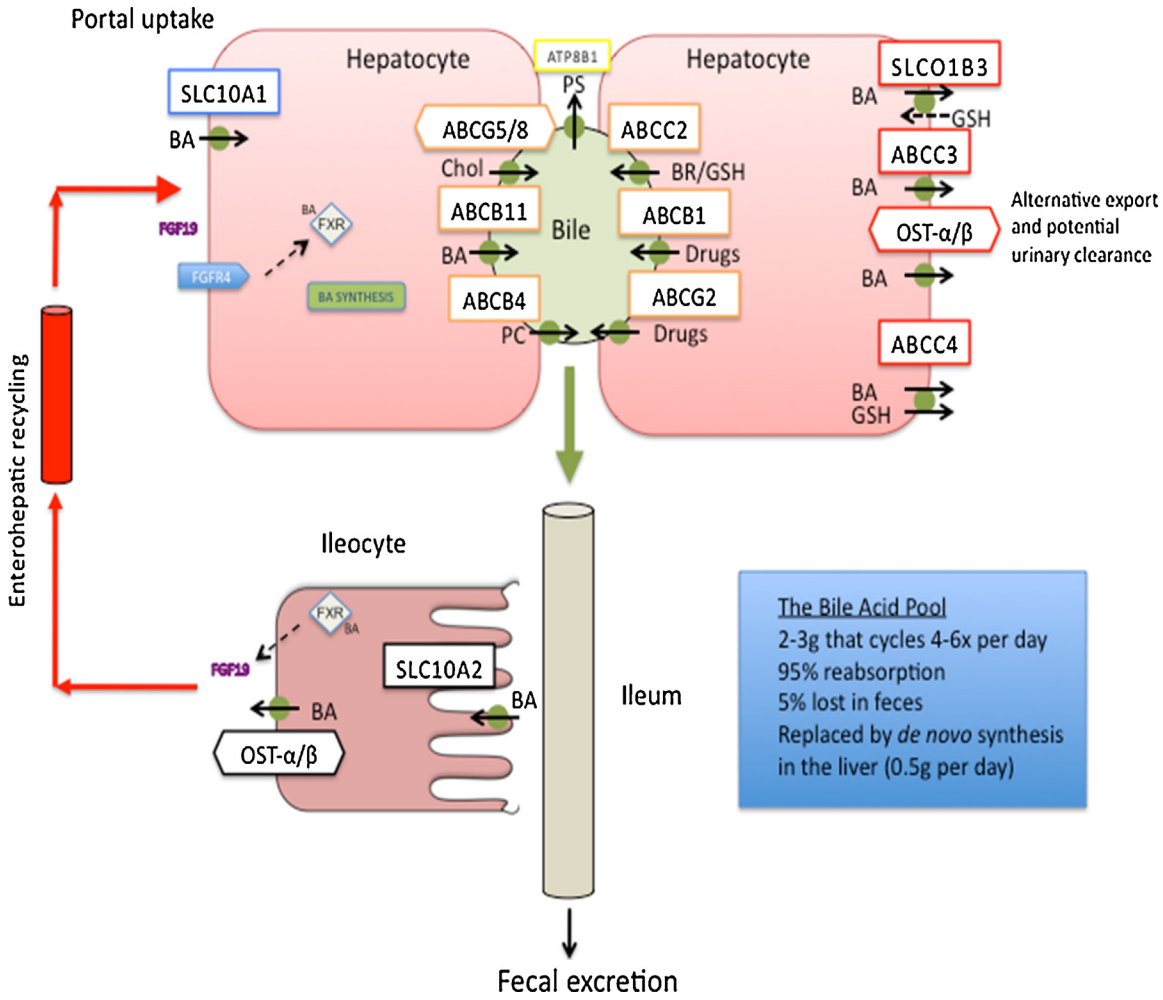

Figure 1 The molecular basis of the enterohepatic circulation of bile acids, showing some of the candidate loci for ICP susceptibility reviewed in this article. Sinusoidal bile acid uptake (>80%) is facilitated by the sodium-dependent taurocholate uptake pump (NTCP, *SLC10A1*) together with *SLCO1B1* (<20%). In addition to BSEP (*ABCB11*), phosphatidyl choline is secreted via MDR3 (*ABCB4*). Other transporters involved in biliary formation and secretion are shown including the multidrug resistance protein 1 (MDR1, *ABCB1*), breast cancer resistance protein (BCRP, *ABCG2*), the sterol transporter complex *ABCG5/8* and the multidrug resistance related protein 2 (MRP2, *ABCC2*), which exports bilirubin. Also shown is *ATP8B1*, a type 4 P-type ATPase, which is also crucial for bile formation and canalicular membrane integrity. The nuclear receptor FXR (NR1H4) is also shown. Reabsorption of bile acids across the ileal enterocyte brush border membrane is via the apical sodium-dependant bile acid transporter ASBT (*SLC10A2*). Following activation of ileocyte FXR and FGF19 expression, export from the ileocyte back into the portal circulation is via the heteromeric transporter OSTa/b. BA: bile acid; Chol: cholesterol; PC: phosphatidyl choline; PS: phosphatidyl serine; BR: bilirubin; GSH: reduced glutathione. For details of genetic studies of these loci see Table 1.

genes include genes responsible for uptake, synthesis and export; hence FXR co-ordinately regulates hepatocellular bile acid levels [24,25].

Bile acid signalling at the cellular surface in some tissues occurs though the membrane-bound G-protein coupled receptor TGR5, resulting in activation of different signalling pathways.

Maternal pathophysiology: genetic susceptibility

Several large pedigrees have been reported where ICP is inherited as a sex-limited dominant phenotype [26,27]. No distinct male phenotype has been identified, although related individuals often have gallstones. Several other lines of evidence indicate a significant genetic component to the pathophysiology, including elevated sibling risk in affected women, and significant variability of disease frequency in different populations (likely due to different genetic background) [28].

Homozygous mutations of some of the hepatobiliary transporters described above were identified as underlying a number of severe paediatric liver diseases, collectively known as progressive familial intrahepatic cholestasis syndromes (PFICs) [29]. Homozygous or compound heterozygous mutations of: *ATP8B1* (a phosphatidyl serine flippase), *ABCB11* (the bile salt export pump) and *ABCB4* (a

phosphatidyl choline floppase) cause PFIC1-3 respectively. A milder form of these diseases, benign recurrent intrahepatic cholestasis (BRIC) has also been described, and there appears to be variable severity of cholestasis in association with different homozygous and heterozygous mutations When mutations of these genes were identified, ICP occurred more frequently than expected. This led to investigations of the role of these genes in ICP.

ABCB4

There have been numerous studies of the canalicular transporter *ABCB4* in ICP. Initial studies were driven by the identification of homozygous mutations of this gene in PFIC [30]. This initial discovery included the observation that mothers of these children, obligate heterozygous carriers of *ABCB4* mutations, had a higher frequency of ICP. This led to the identification of an ICP pedigree in the absence of PFIC with a segregating mutation [31], and subsequently the identification of the first sporadic case of ICP caused by a heterozygous mutation [32].

A considerable number of studies have expanded the range of mutant alleles in this gene associated with ICP (Table 1). Of note, heterozygous mutations of *ABCB4* are implicated in a spectrum of cholestatic liver disease, including inherited diseases such as low-phospholipid associated cholelithiasis (LPAC) syndrome [33], and acquired cholestasis such as drug-induced liver injury. Mutations have also been reported in young women who have not been pregnant following onset of cholestasis induced by the use of hormonal contraception [34].

In addition to rare variants, a common variant in close proximity to the splice site but not altering the protein sequence, rs2109505, has been found to be associated with ICP [35]. In a recent whole-genome sequencing analysis, the same variant was found to be associated with gallstone disease [36]. In the same study, imputed genotypes of 266 cases of ICP and 422 relatives identified several rare variants of *ABCB4* specific to this population and some of the common single nucleotide polymorphisms (SNPs) believed to be associated with ICP in studies in other European populations. These common population variants are not mutations; they are believed to have a minor effect on protein function hence alter risk to a small degree.

In vivo studies of mutant proteins have proved challenging for this gene but recently several groups have reported successful expression of the protein in different cell line systems and characterisation of the effect of specific point mutations, the most recent study being a comprehensive classification of the effect of a series of such mutations [37—40]. These approaches are of particular relevance to ICP and to all of the *ABCB4*-related pathologies as phenotype/genotype relationships are central to an understanding of how mutations cause such a spectrum of phenotypes. In addition, disease associated mutations may have an effect on treatment response.

ABCB11

Heterozygous mutations of this transporter have been recognised as having a smaller but important role in the aetiology of ICP. Several studies have identified both novel and recurrent mutations in ICP cohorts [41—43]. The relatively common Caucasian PFIC2 mutations (E297G and D482G) have also been identified. Based on studies published to date, 5% or more ICP cases may harbour a mutation in this gene (Table 1). As with ABCB4, functional studies, mini-gene construct analysis and immunohistochemistry have been performed to delineate genotype/phenotype correlations [44—46].

The common valine 444 alanine (V444A) polymorphism (rs2287622) has been investigated in ICP [41] along with a number of other liver diseases. Clear evidence exists for an association with ICP although the most recent analysis identified a stronger association from a nearby marker (rs7577650), suggesting that this SNP may be in linkage disequilibrium with the underlying variant; further studies are needed to confirm or refute this. A second, independent association was uncovered at this locus with another SNP (rs3815676) but at a much lower frequency [35]. As with the *ABCB4* variant rs2109505 these changes are not mutations and may only have a small effect on mRNA transcription or protein function and hence a small effect on disease risk.

Other loci

A number of other canalicular transporters are involved in bile formation and membrane stability in hepatocytes as described above. Studies suggest a possible role for *ATP8B1* mutations in limited cases [47,48], and a possible role for *ABCC2* in a South American cohort [49], although the *ABCC2* findings were not replicated in a larger European cohort.[35] As described above, FXR is the key homeostatic sensor of bile acid levels in hepatocytes. Genetic variation at and around this gene has been examined in ICP cohorts and a number of variants identified with functional effects [50]. However, the rare frequency of these alleles implies only a small contribution to overall population susceptibility to ICP.

In addition to these, many other loci have been examined, usually in small studies with low power to detect anything other than near Mendelian like effects (Table 2). No robust evidence exists to date for the involvement of any other loci. However a recent novel population-genetics approach (admixture mapping) may be successful in identifying new genomic regions of interest [51].

Maternal pathophysiology: pruritus

The pruritus associated with ICP can be severely distressing and result in excoriations in an attempt to relieve the symptoms. Previously the bile acids themselves were considered to be a possible pruritic agent, but the levels seen in ICP do not correlate well with self-reported itch scores. However the identification of a TGR5-mediated bile-acid induced signalling pathway has been demonstrated in sensory nerves [52]. Progesterone sulphates were recently shown to correlate with the severity of pruritus in ICP, in addition to affecting TGR5-dependent scratch responses [53].

Another candidate pruritogen is lysophosphatidic acid, a serum lipid involved in a number of signalling pathways and produced by the action of the enzyme autotaxin. Identified

Table 1 Genetic studies of ICP; genes that influence biliary transport.

Gene name	Protein	Cohort studied	Ethnicity	Results and notes	Reference
ABCB4	Multidrug resistance 3 protein (MDR3), flops phosphatidyl choline into lumen	Single large pedigree	French	Co-existence of PFIC3 and ICP, identification of 1712delT in heterozygous mothers	[31]
		8 cases sequenced for entire gene	Caucasian	Single mutation identified, A546D	[32]
		57 cases screened for 1712delT mutation	Finnish	Mutation reported in single French family not seen in Finnish cohort	[90]
		14 cases (entire gene sequenced) plus screening for known variation in 170 further cases	Caucasian	R150K variant identified in one family and extra unrelated case. SNP also found to be associated with ICP	[91]
		Single case of cholelithiasis, cholestasis of pregnancy and biliary cirrhosis	Caucasian	Heterozygous mutation identified, D535G, transmitted to daughter who had ICP	[92]
		20 cases screened for 5 selected exons of *ABCB4*	Caucasian	Single mutations identified, R144X	[93]
		80 cases plus 80 controls screened for mutations of exon 14	Italian	Three heterozygote variants identified (E528D, R549H, G536R)	[94]
		Single large pedigree	Mennonite	54bp in-frame deletion caused by cryptic splice site activation identified	[95]
		Above study extended to 96 women and three exons screened	Italian	Three further variants identified (R590Q, R652G and T667I)	[96]
		10 cases with raised GGT	Italian	Novel splicing mutation and recurrent missense mutation(R590Q) identified	[97]
		Cohort of 50 ICP cases sequenced	Caucasian	Eight mutations identified (R144X, S320F, T775M and five cases of R590Q)	[98]
		59 patents (mix of ICP and CIC) sequenced, large indels also analysed	French	16 point mutations/small indels identified and a single whole-gene heterozygous deletion in CIC case	[99]
		Sequencing of a large LPAC cohort including ICP cases	French	Six truncation mutations and 17 missense mutations identified in ICP part of cohort	[100]
		Single case of severe recurrent early onset ICP	Hispanic	Heterozygous mutation identified (W164G)	[101]
		Single case of recurrent gallstones and ICP	NK	Heterozygous mutation identified (P726L)	[102]
		Large cohort of adult patients including 4 with ICP sequenced	Italian	Two heterozygous mutations identified (L859W and S320F)	[103]

Table 1 (*Continued*)

Gene name	Protein	Cohort studied	Ethnicity	Results and notes	Reference
ABCB11	Bile salt export pump (BSEP), exports monovalent bile salts into lumen	57 cases and 115 controls typed for two SNPs	Finnish	Single locus SNP plus haplotype analysis showed evidence of association	[104]
		142 cases and two control groups of 100 each	Finnish	Failed to reproduce above association	[105]
		491 cases screened for five known mutant alleles and typed for V444A polymorphism	Caucasian	E297G mutation identified in four cases, D482 once and N591S twice. The 444A SNP was found to be associated with ICP	[42]
ABCC2	Multidrug resistance related protein 2 (MRP2); exports organic anions including bilirubin into bile	70 cases and 112 controls analysed four 6 SNPs	South American	Association between rs3740066 in exon 28 and ICP	[49]
ATP8B1	Familial intrahepatic cholestasis 1 gene (FIC1), flips phosphatidyl serine into hepatocyte from lumen	Sixteen cases sequenced, variants detected then analysed in 182 patients and 120 controls	Caucasian	D70N found in three cases. R867C found in a single case. F305I seen in a single case and single control	[48]
		Linkage suggested involvement of loci in four families. 176 cases screened	Finnish	17 sequence changes detected, two novel missense mutations (N45T, K203R) suggested to predispose to ICP	[47]
NR1H4	Farnesoid-X receptor (FXR), master regulator of bile acid homeostasis	92 cases sequenced, subsequent case-control study of 293 cases and 290 controls plus 49 cases and 59 controls	Caucasian and mixed	Four novel heterozygous variants identified (-1g>t, M1V, W80R, M173T). M173T associated with ICP in combined analysis, functional defects shown for 3 variants	[50]
		6 cases sequenced together with 2 drug-induced cholestasis cases	NK	Single heterozygous variant identified (-1g>t)	[106]
NR1I2	Pregnane-X receptor (PXR), sensor of xenobiotics	Sequencing of entire gene in 121 cases, exon 2 in a further 226 cases	Caucasian	Polymorphisms identified at identical frequencies in cases and controls	[107]
		4 tag SNPs plus three other functional variants typed in 101 cases and 171	South America	Association reported between rs2461823 and ICP	[108]
Multiple loci analysed					
	ABCB4, ABCB11, AT8B1	16 individuals from two Finnish ICP families	Finnish	Linkage analysis (plus *ABCB4* sequencing) to exclude 3 loci	[109]

Table 1 (*Continued*)

Multiple loci analysed					
	ABCB4, ABCB11	21 cases and 40 controls sequenced for coding region of two genes	Caucasian	Single *ABCB11* variant (N591S) and two *ABCB4* variants (S320F and G762E) plus four ABCB4 splicing mutations identified	[41]
	ABCB4, ABCB11	Single severe case sequenced for both genes	Moroccan	Combination of homozygous *ABCB4* mutation (S320F) and V444A *ABCB11* polymorphism identified	[110]
	ABCB4, ABCB11	Haplotype study of 52 cases and 52 controls	Caucasian	Single haplotype of *ABCB4* seen more commonly in cases and two seen more commonly in controls. *ABCB11* haplotypes showed no differences in distribution	[111]
	ABCB11, ABCC2	Coding SNP of *ABCB11* and two coding SNPs of *ABCC2* analysed in 42 and 33 cases respectively	Caucasian	V444A polymorphism of *ABCB11* associated with ICP	[112]
	ABCB4, ABCB11	Single case of recurrent ICP and choledocholithiasis	NK	Heterozygous stop codon in *ABCB4* and V444A polymorphism identified	[113][a]
	ABCB4, ABCB11, ATP8B1	Sequencing of single severe case	French	V444A polymorphism of *ABCB11* found in homozygous state	[114]
	ABCB4, ABCB11, NR1H4	Sequencing of single case for ABCB4 and ABCB11 and typing for 5 NR1H4 SNPs	NK	Heterozygous mutation in ABCB4 (S320F) together with V444A in ABCB11 and -1g>t in NR1H4	[115]
	ABCB4, ABCB11, ABCG8	Single case with marked hepatocellular dysfunction	NK	R590Q identified in *ABCB4* together with the 444A SNP in *ABCB11* and D19H in *ABCG8*	[116]
	ABCB4, ABCB11	33 cases sequenced	Italian	Five *ABCB4* mutations identified (L73V, T175A, N510S, a splice site mutation and an insertion causing frame shift). Six ABCB11 variants identified (E135K, V284D, D482G, Q558H, R698H, P731S)	[43]
	ABCB4, ABCB11, ABCC2, ATP8B1, NR1H4, FGF19	SNP analysis around each locus in 563 cases and 642 controls. Findings confirmed in second cohort of 227 cases	Caucasian	Strong association signals with two SNPs in *ABCB11* (rs3815676, rs7577650) and one in *ABCB4* (rs2109505)	[35]

NK: not known.
[a] Abstract only available in English.

Table 2 Genetic studies of ICP; genes that do not influence biliary transport.

Gene or locus name	Protein	Cohort studied	Ethnicity	Results and notes	Reference
HLA-DPB1	Human leucocyte antigen	26 cases and 30 controls	Chilean	No association of disease found with HLA-DPB1 alleles detected by PCR/SSOH	[117]
ApoE	Apolipoprotein E	44 cases and 47 controls	Finnish	No differences in apoE allele distribution	[118]
HLA-DRB1	Human leucocyte antigen	42 cases, 56 controls	Chinese	Over-representation of DR6 allele in patient group, suggested as ICP susceptibility locus	[119][a]
ER alpha	Estrogen receptor alpha	57 cases, 47 controls	Finnish	No differences in genotype distribution of two intronic ER alpha polymorhphisms	[90]
2p13	—	45 cases and 47 controls	Finnish	Two markers from the 2p13 region associated with ICP	[120]
2p13	—	57 cases, 133 PET cases, 115 controls	Finnish	Expanded above study and reported common risk locus for ICP and PET	[121]
HLA-DPA1	Human leucocyte antigen	25 families and 25 control families	Chinese	No differences in frequencies of HLA-DPA1 alleles observed	[122][a]
ER alpha	Estrogen receptor alpha	100 cases, 100 controls	Chinese	No differences in genotype frequencies of two intronic polymorphisms between cases and controls	[123][a]
ESR2	Estrogen receptor 2	100 cases and 100 controls	Chinese	Two RFLPs studied (exons 5 and 8) and exon 8 polymorphism associated with risk of ICP	[124][a]
CYP17 and *CYP3A4*	Cytochrome P450 enzymes involved in estrogen metabolism	100 cases and 100 controls	Chinese	Promoter polymorphism of CYP17 not associated with OC, single CYP3A4 promoter polymorphism not observed in this population	[125][a]
HLA-DQA1	Human leucocyte antigen	45 cases, 45 controls plus 18 families and eighteen control families	Chinese	No association between OC and HLA-DQA1; HLA-DQA1*0301 proposed as a protective gene	[126][a]
HLA-G	Human leucocyte antigen	30 cases and offspring, 30 controls and offspring	Chinese	No association with 14bp deletion polymorphism of HLA-G and ICP	[127][a]
ACTG2	Gamma 2 actin gene	57 cases and 115 controls	Finnish	No differences in distribution of ACTG2 intron 1 indel polymorphism	[128]
CYP1A1	Cytochrome P450	100 cases and 100 controls	Chinese	Exon 7 I/V polymorphism proposed to be associated with ICP	[129][a]
CYP1B1	Cytochrome P450	100 cases and 100 controls	Chinese	Polymorphism of exon 2 proposed to be associated with ICP	[130][a]
ER beta	Estrogen receptor beta	Two groups of 105 cases and 105 controls	Chinese (Han and Uygurs)	Propose different polymorphisms associated with ICP in different ethnic groups	[131][a]
RAGE, GLO I	Receptor for advanced glycation, glyoxalase I enzyme	120 controls and 14 ICP cases	Czech	No associations detected with 4 and 1 polymorphism in the two respective genes	[132]
Chromosome 2	Over 40 genes in candidate region	198 cases and 174 controls	USA and Chile	Admixture mapping	[51]

PET: pre-eclampsia.
[a] Only the abstract of the article is available in English.

as a mediator of cholestatic pruritus [54], it may be clinically useful as a new diagnostic marker [55].

Maternal pathophysiology: reproductive hormone involvement

Maternal metabolic changes are key to a successful pregnancy. However, these adaptations can become pathological in genetically susceptible individuals, resulting in gestational cholestasis or diabetes mellitus. Hence, another component to understanding the aetiology of ICP is the role of reproductive hormones. With respect to ICP, serum levels of estrogen and progesterone are highest during the third trimester when the disease usually presents.

A number of rodent studies have shown a cholestatic effect of estrogen administration, with effects seen on transporter expression and localization [56—59]. Studies of hepatic expression profiles using microarrays in Fxr knockout mice compared to pregnant controls indicated similar changes, suggesting a desensitization of the FXR pathway in normal pregnancy and *in vitro* studies indicate this may be estrogen-dependent [60]. It was recently demonstrated that FXR can be directly inhibited by 17β-estradiol-activated estrogen receptor α, resulting in an inhibition of the transcription of ABCB11 [61].

In addition to estrogen, progesterone is of considerable importance in the aetiology of ICP. In particular, a number of progesterone metabolites have been identified as capable of cross-talk with bile acid signalling pathways, thus having an impact on the cholestatic phenotype. These metabolites are raised in normal pregnancy and further elevated in ICP (reviewed in [62]). They have been shown to impact hepatocellular bile acid influx by competitively inhibiting NTCP, and to reduce BSEP-mediated efflux [63,64]. Certain metabolites also act as partial agonists of FXR, contributing to the cholestatic phenotype by desensitising FXR-regulated pathways [65]. Further, specific metabolites may serve as early predictive biomarkers as they are elevated prior to increased serum bile acids [53].

Placental and fetal pathophysiology

During gestation the placenta has a key feto-protective role that includes limiting exposure to endobiotic toxic compounds such as bile acids [66]. However, the pathways and signalling involved are considerably different to those in hepatocytes. Nuclear receptors key to hepatic bile acid homeostasis are expressed at low levels [67] and the key bile acid transporter ABCB11 is not expressed. It is likely that another ATP-dependant transporter, ABCG2, mediates placental bile acid efflux [68].

Several studies have reported changes in placental morphology, although these results were not confirmed in one other study [69—72]. Studies of specific pathways have identified a number of changes in cholestatic placentas, including hypoxia-regulated genes [73], urocortin [74], PPAR-gamma/NF-kappa beta pathways [75] and 11betaHSD2 [76]. A single proteomic analysis and a single expression array analysis both produced a range of alterations [77,78] but as yet a clear pattern of the effect of cholestasis on the placenta has not been established. TGR5 is expressed in trophoblast cells and fetal macrophages and hence cholestasis may affect the placenta through these pathways [79]. A dual perfusion ex-vivo model of ICP suggested a direct effect of taurocholic acid on the placental vasculature, which could contribute to placental dysfunction and adverse fetal outcomes [80].

Although the adverse fetal outcomes are the major concern in the clinical management of ICP, they are poorly understood. Evidence is accumulating for bile-acid induced arrhythmias playing a role [81] and placental dysfunction caused by bile acid damage (see above) may also contribute [71]. Bile acids may be responsible for increased rates of preterm labour via prostagladin pathways and may stimulate fetal gut motility resulting in meconium-stained amniotic fluid, although this may be a secondary effect of bile acid toxicity [82,83]. Murine studies also support a role for bile acids in disrupting pulmonary surfactant in neonates, potentially leading to the respiratory distress seen in ICP [84].

Future perspectives

Our current understanding of ICP is that the elevated levels of reproductive hormones unmask genetic susceptibility in some women, resulting in cholestasis and elevated serum bile acids.

A mechanistic understanding of the adverse fetal outcomes, and the programming effects of *in utero* exposure to high bile acids remain to be established.

The bacterial content of the gut, the microbiome, is being increasingly recognised as influencing gut-liver signalling. Pregnancy itself has a profound effect on the composition of the microbiome [85] and cholestasis in rodent models has been demonstrated to have a considerable impact [86]. Thus, a role for an altered microbiome in ICP seems likely. In addition epigenetic alterations, which represent an attractive mechanism for contributing to maternal and fetal pathophysiology have only to date been investigated in a single study of methylation of white blood cell promotors in ICP women [87]. Further genomic-driven insights seem likely, as we have now entered the era of whole genome sequencing (WGS) [36,88]; analysis of an ICP cohort using this approach is awaited.

Therapeutic options may also be expanded in the future as a range of new therapies targeted at different components of the enterohepatic circulation comes to market [89]. It is hoped that sophisticated genomic approaches, in combination with robust evaluation of therapies, will enable a more personalised approach to be adopted in the future management of ICP.

Disclosure of interest

The authors declare that they have no competing interest.

Contribution

The authors jointly prepared the article.

Acknowledgments

Research in the Williamson lab is supported by the Wellcome Trust (P30874), the MRC, the Lauren Page Trust, ICP Support,

FP7 programme Marie Curie Actions and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

References

[1] Reyes H, Gonzalez MC, Ribalta J, Aburto H, Matus C, Schramm G, et al. Prevalence of intrahepatic cholestasis of pregnancy in Chile. Ann Intern Med 1978;88:487—93.
[2] Reyes H. Sex hormones and bile acids in intrahepatic cholestasis of pregnancy. Hepatology 2008;47:376—9.
[3] Geenes V, Williamson C. Intrahepatic cholestasis of pregnancy. World J Gastroenterol 2009;15:2049—66.
[4] Kenyon AP, Piercy CN, Girling J, Williamson C, Tribe RM, Shennan AH. Obstetric cholestasis, outcome with active management: a series of 70 cases. BJOG 2002;109:282—8.
[5] Williamson C, Geenes V. Intrahepatic cholestasis of pregnancy. Obstet Gynecol 2014;124:120—33.
[6] Lammert F, Marschall HU, Matern S. Intrahepatic cholestasis of pregnancy. Curr Treat Options Gastroenterol 2003;6:123—32.
[7] Walker IA, Nelson-Piercy C, Williamson C. Role of bile acid measurement in pregnancy. Ann Clin Biochem 2002;39:105—13.
[8] Dann AT, Kenyon AP, Wierzbicki AS, Seed PT, Shennan AH, Tribe RM. Plasma lipid profiles of women with intrahepatic cholestasis of pregnancy. Obstet Gynecol 2006;107:106—14.
[9] Martineau MG, Raker C, Dixon PH, Chambers J, Machirori M, King NM, et al. The metabolic profile of intrahepatic cholestasis of pregnancy is associated with impaired glucose tolerance, dyslipidemia, and increased fetal growth. Diabetes Care 2015;38:243—8.
[10] Goulis DG, Walker IA, de Swiet M, Redman CW, Williamson C. Preeclampsia with abnormal liver function tests is associated with cholestasis in a subgroup of cases. Hypertens Pregnancy 2004;23:19—27.
[11] Martineau M, Raker C, Powrie R, Williamson C. Intrahepatic cholestasis of pregnancy is associated with an increased risk of gestational diabetes. Eur J Obstet Gynecol Reprod Biol 2014;176:80—5.
[12] Fisk NM, Storey GN. Fetal outcome in obstetric cholestasis. Br J Obstet Gynaecol 1988;95:1137—43.
[13] Williamson C, Hems LM, Goulis DG, Walker I, Chambers J, Donaldson O, et al. Clinical outcome in a series of cases of obstetric cholestasis identified via a patient support group. BJOG 2004;111:676—81.
[14] Glantz A, Marschall HU, Mattsson LA. Intrahepatic cholestasis of pregnancy: relationships between bile acid levels and fetal complication rates. Hepatology 2004;40:467—74.
[15] Geenes V, Chappell LC, Seed PT, Steer PJ, Knight M, Williamson C. Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study. Hepatology 2014;59:1482—91.
[16] Geenes V, Lovgren-Sandblom A, Benthin L, Lawrance D, Chambers J, Gurung V, et al. The reversed feto-maternal bile acid gradient in intrahepatic cholestasis of pregnancy is corrected by ursodeoxycholic acid. PLoS One 2014;9:e83828.
[17] Bacq Y, Sentilhes L, Reyes HB, Glantz A, Kondrackiene J, Binder T, et al. Efficacy of ursodeoxycholic acid in treating intrahepatic cholestasis of pregnancy: a meta-analysis. Gastroenterology 2012;143:1492—501.
[18] Geenes V, Chambers J, Khurana R, Shemer EW, Sia W, Mandair D, et al. Rifampicin in the treatment of severe intrahepatic cholestasis of pregnancy. Eur J Obstet Gynecol Reprod Biol 2015;189:59—63.
[19] Liver EAftSot. EASL Clinical Practice Guidlines: management of cholestatic liver diseases. J Hepatol 2009;51:237—67.
[20] Wikstrom Shemer E, Marschall HU, Ludvigsson JF, Stephansson O. Intrahepatic cholestasis of pregnancy and associated adverse pregnancy and fetal outcomes: a 12-year population-based cohort study. BJOG 2013;120:717—23.
[21] Wikstrom Shemer EA, Stephansson O, Thuresson M, Thorsell M, Ludvigsson JF, Marschall H-U. Intrahepatic cholestasis of pregnancy and cancer, immune-mediated and cardiovascular diseases: a population-based cohort study. J Hepatol 2015;63:456—61.
[22] Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, Briz O, Owen BM, Nikolova V, et al. Maternal cholestasis during pregnancy programs metabolic disease in offspring. J Clin Invest 2013;123:3172—81.
[23] Boyer JL. Bile formation and secretion. Compr Physiol 2013;3:1035—78.
[24] Mazuy C, Helleboid A, Staels B, Lefebvre P. Nuclear bile acid signaling through the farnesoid X receptor. Cell Mol Life Sci 2015;72:1631—50.
[25] Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap — bile acids in metabolic control. Nat Rev Endocrinol 2014;10:488—98.
[26] Reyes H, Ribalta J, Gonzalez-Ceron M. Idiopathic cholestasis of pregnancy in a large kindred. Gut 1976;17:709—13.
[27] Holzbach RT, Sivak DA, Braun WE. Familial recurrent intrahepatic cholestasis of pregnancy: a genetic study providing evidence for transmission of a sex-limited, dominant trait. Gastroenterology 1983;85:175—9.
[28] Palmer DG, Eads J. Intrahepatic cholestasis of pregnancy: a critical review. J Perinat Neonatal Nurs 2000;14:39—51.
[29] van der Woerd WL, van Mil SW, Stapelbroek JM, Klomp LW, van de Graaf SF, Houwen RH. Familial cholestasis: progressive familial intrahepatic cholestasis, benign recurrent intrahepatic cholestasis and intrahepatic cholestasis of pregnancy. Best practice & research. Clin Gastroenterol 2010;24:541—53.
[30] de Vree JM, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, Aten J, et al. Mutations in the *MDR3* gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci U S A 1998;95:282—7.
[31] Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. Heterozygous non-sense mutation of the *MDR3* gene in familial intrahepatic cholestasis of pregnancy. Lancet 1999;353:210—1.
[32] Dixon PH, Weerasekera N, Linton KJ, Donaldson O, Chambers J, Egginton E, et al. Heterozygous MDR3 missense mutation associated with intrahepatic cholestasis of pregnancy: evidence for a defect in protein trafficking. Hum Mol Genet 2000;9:1209—17.
[33] Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the *MDR3/ABCB4* gene. Orphanet J Rare Dis 2007;2:29.
[34] Jirsa M, Bronsky J, Dvorakova L, Sperl J, Smajstrla V, Horak J, et al. ABCB4 mutations underlie hormonal cholestasis but not pediatric idiopathic gallstones. World J Gastroenterol 2014;20:5867—74.
[35] Dixon PH, Wadsworth CA, Chambers J, Donnelly J, Cooley S, Buckley R, et al. A comprehensive analysis of common genetic variation around six candidate loci for intrahepatic cholestasis of pregnancy. Am J Gastroenterol 2014;109:76—84.
[36] Gudbjartsson DF, Helgason H, Gudjonsson SA, Zink F, Oddson A, Gylfason A, et al. Large-scale whole-genome sequencing of the Icelandic population. Nat Genet 2015;47:435—44.

[37] Andress EJ, Nicolaou M, Romero MR, Naik S, Dixon PH, Williamson C, et al. Molecular mechanistic explanation for the spectrum of cholestatic disease caused by the S320F variant of ABCB4. Hepatology 2014;59:1921—31.
[38] Gautherot J, Delautier D, Maubert M-A, Ait-Slimane T, Bolbach G, Delaunay J-L, et al. Phosphorylation of ABCB4 impacts its function: insights from disease-causing mutations. Hepatology 2014;60:610—21.
[39] Gordo-Gilart R, Andueza S, Hierro L, Martinez-Fernandez P, D'Agostino D, Jara P, et al. Functional analysis of ABCB4 mutations relates clinical outcomes of progressive familial intrahepatic cholestasis type 3 to the degree of MDR3 floppase activity. Gut 2015;64:147—55.
[40] Delaunay JL, Durand-Schneider AM, Dossier C, Falguieres T, Gautherot J, Davit-Spraul A, et al. A functional classification of ABCB4 variations causing progressive familial intrahepatic cholestasis type 3. Hepatology 2015, http://dx.doi.org/10.1002/hep.28300 [E-pub ahead of print].
[41] Pauli-Magnus C, Lang T, Meier Y, Zodan-Marin T, Jung D, Breymann C, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. Pharmacogenetics 2004;14:91—102.
[42] Dixon PH, van Mil SW, Chambers J, Strautnieks S, Thompson RJ, Lammert F, et al. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. Gut 2009;58:537—44.
[43] Anzivino C, Odoardi MR, Meschiari E, Baldelli E, Facchinetti F, Neri I, et al. ABCB4 and ABCB11 mutations in intrahepatic cholestasis of pregnancy in an Italian population. Dig Liver Dis 2013;45:226—32.
[44] Strautnieks SS, Byrne JA, Pawlikowska L, Cebecauerova D, Rayner A, Dutton L, et al. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology 2008;134:1203—14.
[45] Byrne JA, Strautnieks SS, Ihrke G, Pagani F, Knisely AS, Linton KJ, et al. Missense mutations and single nucleotide polymorphisms in ABCB11 impair bile salt export pump processing and function or disrupt pre-messenger RNA splicing. Hepatology 2009;49:553—67.
[46] Noe J, Stieger B, Meier PJ. Functional expression of the canalicular bile salt export pump of human liver. Gastroenterology 2002;123:1659—66.
[47] Painter JN, Savander M, Ropponen A, Nupponen N, Riikonen S, Ylikorkala O, et al. Sequence variation in the *ATP8B1* gene and intrahepatic cholestasis of pregnancy. Eur J Hum Genet 2005;13:435—9.
[48] Mullenbach R, Bennett A, Tetlow N, Patel N, Hamilton G, Cheng F, et al. ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. Gut 2005;54:829—34.
[49] Sookoian S, Castano G, Burgueno A, Gianotti TF, Pirola CJ. Association of the multidrug-resistance-associated protein gene (ABCC2) variants with intrahepatic cholestasis of pregnancy. J Hepatol 2008;48:125—32.
[50] Van Mil SW, Milona A, Dixon PH, Mullenbach R, Geenes VL, Chambers J, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. Gastroenterology 2007;133:507—16.
[51] Bull LN, Hu D, Shah S, Temple L, Silva K, Huntsman S, et al. Intrahepatic cholestasis of pregnancy (ICP) in U.S. Latinas and Chileans: clinical features, ancestry analysis, and admixture mapping. PloS One 2015;10:e0131211.
[52] Alemi F, Kwon E, Poole DP, Lieu T, Lyo V, Cattaruzza F, et al. The TGR5 receptor mediates bile acid-induced itch and analgesia. J Clin Investig 2013;123:1513—30.
[53] Abu-Hayyeh S, Ovadia C, Lieu T, Jensen DD, Chambers J, Dixon PH, et al. Prognostic and mechanistic potential of progesterone sulfates in intrahepatic cholestasis of pregnancy and pruritus gravidarum. Hepatology 2015, http://dx.doi.org/10.1002/hep.28265 [E-pub ahead of print].
[54] Kremer AE, Martens JJ, Kulik W, Rueff F, Kuiper EM, van Buuren HR, et al. Lysophosphatidic acid is a potential mediator of cholestatic pruritus. Gastroenterology 2010;139, 1008-18, 18 e1.
[55] Kremer AE, Bolier R, Dixon PH, Geenes V, Chambers J, Tolenaars D, et al. Autotaxin activity has a high accuracy to diagnose intrahepatic cholestasis of pregnancy. J Hepatol 2015;62:897—904.
[56] Crocenzi FA, Mottino AD, Cao J, Veggi LM, Pozzi EJ, Vore M, et al. Estradiol-17beta-D-glucuronide induces endocytic internalization of Bsep in rats. Am J Physiol Gastrointest Liver Physiol 2003;285:G449—59.
[57] Geier A, Dietrich CG, Gerloff T, Haendly J, Kullak-Ublick GA, Stieger B, et al. Regulation of basolateral organic anion transporters in ethinylestradiol-induced cholestasis in the rat. Biochim Biophys Acta 2003;1609:87—94.
[58] Simon FR, Fortune J, Iwahashi M, Qadri I, Sutherland E. Multihormonal regulation of hepatic sinusoidal *Ntcp* gene expression. Am J Physiol Gastrointest Liver Physiol 2004;287:G782—94.
[59] Yamamoto Y, Moore R, Hess HA, Guo GL, Gonzalez FJ, Korach KS, et al. Estrogen receptor alpha mediates 17alpha-ethynylestradiol causing hepatotoxicity. J Biol Chem 2006;281:16625—31.
[60] Milona A, Owen BM, Cobbold JF, Willemsen EC, Cox IJ, Boudjelal M, et al. Raised hepatic bile acid concentrations during pregnancy in mice are associated with reduced farnesoid X receptor function. Hepatology 2010;52:1341—9.
[61] Song X, Vasilenko A, Chen Y, Valanejad L, Verma R, Yan B, et al. Transcriptional dynamics of bile salt export pump during pregnancy: mechanisms and implications in intrahepatic cholestasis of pregnancy. Hepatology 2014;60:1993—2000.
[62] Reyes H, Sjovall J. Bile acids and progesterone metabolites in intrahepatic cholestasis of pregnancy. Ann Med 2000;32:94—106.
[63] Abu-Hayyeh S, Martinez-Becerra P, Sheikh Abdul Kadir SH, Selden C, Romero MR, Rees M, et al. Inhibition of Na+-taurocholate Co-transporting polypeptide-mediated bile acid transport by cholestatic sulfated progesterone metabolites. J Biol Chem 2010;285:16504—12.
[64] Vallejo M, Briz O, Serrano MA, Monte MJ, Marin JJ. Potential role of trans-inhibition of the bile salt export pump by progesterone metabolites in the etiopathogenesis of intrahepatic cholestasis of pregnancy. J Hepatol 2006;44:1150—7.
[65] Abu-Hayyeh S, Papacleovoulou G, Lovgren-Sandblom A, Tahir M, Oduwole O, Jamaludin NA, et al. Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites inhibit farnesoid X receptor resulting in a cholestatic phenotype. Hepatology 2013;57:716—26.
[66] Marin JJ, Macias RI, Briz O, Perez MJ, Serrano MA. Molecular bases of the excretion of fetal bile acids and pigments through the fetal liver-placenta-maternal liver pathway. Ann Hepatol 2005;4:70—6.
[67] Geenes VL, Dixon PH, Chambers J, Raguz S, Marin JJ, Bhakoo KK, et al. Characterisation of the nuclear receptors FXR, PXR and CAR in normal and cholestatic placenta. Placenta 2011;32:535—7.
[68] Blazquez AG, Briz O, Romero MR, Rosales R, Monte MJ, Vaquero J, et al. Characterization of the role of ABCG2 as a bile acid transporter in liver and placenta. Mol Pharmacol 2012;81:273—83.
[69] Costoya AL, Leontic EA, Rosenberg HG, Delgado MA. Morphological study of placental terminal villi in intrahepatic

cholestasis of pregnancy: histochemistry, light and electron microscopy. Placenta 1980;1:361—8.
[70] Wikstrom Shemer E, Thorsell M, Ostlund E, Blomgren B, Marschall HU. Stereological assessment of placental morphology in intrahepatic cholestasis of pregnancy. Placenta 2012;33:914—8.
[71] Geenes VL, Lim YH, Bowman N, Tailor H, Dixon PH, Chambers J, et al. A placental phenotype for intrahepatic cholestasis of pregnancy. Placenta 2011;32:1026—30.
[72] Patel S, Pinheiro M, Felix JC, Opper N, Ouzounian JG, Lee RH. A case-control review of placentas from patients with intrahepatic cholestasis of pregnancy. Fetal Pediatr Pathol 2014;33:210—5.
[73] Wei W, Hu YY. Expression of hypoxia-regulated genes and glycometabolic genes in placenta from patients with intrahepatic cholestasis of pregnancy. Placenta 2014;35:732—6.
[74] Zhou F, Zhang L, Sun Q, Wang XD. Expression of urocortin and corticotrophin-releasing hormone receptor-2 in patients with intrahepatic cholestasis of pregnancy. Placenta 2014;35:962—8.
[75] Zhang Y, Hu L, Cui Y, Qi Z, Huang X, Cai L, et al. Roles of PPARgamma/NF-kappaB signaling pathway in the pathogenesis of intrahepatic cholestasis of pregnancy. PLoS One 2014;9:e87343.
[76] Martineau M, Papacleovoulou G, Abu-Hayyeh S, Dixon PH, Ji H, Powrie R, et al. Cholestatic pregnancy is associated with reduced placental 11betaHSD2 expression. Placenta 2014;35:37—43.
[77] He P, Wang F, Jiang Y, Zhong Y, Lan Y, Chen S. Placental proteome alterations in women with intrahepatic cholestasis of pregnancy. Int J Gynaecol Obstet 2014;126:256—9.
[78] Du Q, Pan Y, Zhang Y, Zhang H, Zheng Y, Lu L, et al. Placental gene-expression profiles of intrahepatic cholestasis of pregnancy reveal involvement of multiple molecular pathways in blood vessel formation and inflammation. BMC Med Genomics 2014;7:42.
[79] Keitel V, Spomer L, Marin JJG, Williamson C, Geenes V, Kubitz R, et al. Effect of maternal cholestasis on TGR5 expression in human and rat placenta at term. Placenta 2013;34:810—6.
[80] Dolinsky BM, Zelig CM, Paonessa DJ, Hoeldtke NJ, Napolitano PG. Effect of taurocholic acid on fetoplacental arterial pressures in a dual perfusion placental cotyledon model: a novel approach to intrahepatic cholestasis of pregnancy. J Reprod Med 2014;59:367—70.
[81] Miragoli M, Kadir SHSA, Sheppard MN, Salvarani N, Virta M, Wells S, et al. A protective antiarrhythmic role of ursodeoxycholic acid in an in vitro rat model of the cholestatic fetal heart. Hepatology 2011;54:1282—90.
[82] Campos GA, Guerra FA, Israel EJ. Effects of cholic acid infusion in fetal lambs. Acta Obstet Gynecol Scand 1986;65:23—6.
[83] Israel EJ, Guzman ML, Campos GA. Maximal response to oxytocin of the isolated myometrium from pregnant patients with intrahepatic cholestasis. Acta Obstet Gynecol Scand 1986;65:581—2.
[84] Zhang Y, Fei L, Wang Y, Pitre A, Fang Z-Z, Frank MW, et al. Maternal bile acid defficiency promotes neonatal demise. Nat Commun 2015;6:8186.
[85] DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A 2015;112:11060—5.
[86] Fouts DE, Torralba M, Nelson KE, Brenner DA, Schnabl B. Bacterial translocation and changes in the intestinal microbiome in mouse models of liver disease. J Hepatol 2012;56:1283—92.
[87] Cabrerizo R, Castano GO, Burgueno AL, Fernandez Gianotti T, Gonzalez Lopez Ledesma MM, Flichman D, et al. methylation of farnesoid X receptor and pregnane X receptor modulates the intrahepatic cholestasis of pregnancy phenotype. PloS One 2014;9:e87697.
[88] Consortium TGP. A global reference for human genetic variation. Nature 2015;526:68—74.
[89] Beuers U, Trauner M, Jansen P, Poupon R. New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. J Hepatol 2015;62:S25—37.
[90] Eloranta ML, Heiskanen JT, Hiltunen MJ, Mannermaa AJ, Punnonen KR, Heinonen ST. Multidrug resistance 3 gene mutation 1712delT and estrogen receptor alpha gene polymorphisms in Finnish women with obstetric cholestasis. Eur J Obstet Gynecol Reprod Biol 2002;105:132—5.
[91] Mullenbach R, Linton KJ, Wiltshire S, Weerasekera N, Chambers J, Elias E, et al. *ABCB4* gene sequence variation in women with intrahepatic cholestasis of pregnancy. J Med Genet 2003;40:e70.
[92] Lucena JF, Herrero JI, Quiroga J, Sangro B, Garcia-Foncillas J, Zabalegui N, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. Gastroenterology 2003;124:1037—42.
[93] Gendrot C, Bacq Y, Brechot MC, Lansac J, Andres C. A second heterozygous MDR3 nonsense mutation associated with intrahepatic cholestasis of pregnancy. J Med Genet 2003;40:e32.
[94] Floreani A, Carderi I, Paternoster D, Soardo G, Azzaroli F, Esposito W, et al. Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. Aliment Pharmacol Ther 2006;23:1649—53.
[95] Schneider G, Paus TC, Kullak-Ublick GA, Meier PJ, Wienker TF, Lang T, et al. Linkage between a new splicing site mutation in the *MDR3* alias *ABCB4* gene and intrahepatic cholestasis of pregnancy. Hepatology 2007;45:150—8.
[96] Floreani A, Carderi I, Paternoster D, Soardo G, Azzaroli F, Esposito W, et al. Hepatobiliary phospholipid transporter *ABCB4*, *MDR3* gene variants in a large cohort of Italian women with intrahepatic cholestasis of pregnancy. Dig Liver Dis 2008;40:366—70.
[97] Tavian D, Degiorgio D, Roncaglia N, Vergani P, Cameroni I, Colombo R, et al. A new splicing site mutation of the *ABCB4* gene in intrahepatic cholestasis of pregnancy with raised serum gamma-GT. Dig Liver Dis 2009;41:671—5.
[98] Bacq Y, Gendrot C, Perrotin F, Lefrou L, Chretien S, Vie-Buret V, et al. *ABCB4* gene mutations and single-nucleotide polymorphisms in women with intrahepatic cholestasis of pregnancy. J Med Genet 2009;46:711—5.
[99] Pasmant E, Goussard P, Baranes L, Laurendeau I, Quentin S, Ponsot P, et al. First description of *ABCB4* gene deletions in familial low phospholipid-associated cholelithiasis and oral contraceptives-induced cholestasis. Eur J Hum Genet 2012;20:277—82.
[100] Poupon R, Rosmorduc O, Boelle PY, Chretien Y, Corpechot C, Chazouilleres O, et al. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. Hepatology 2013;58:1105—10.
[101] Johnston RC, Stephenson ML, Nageotte MP. Novel heterozygous *ABCB4* gene mutation causing recurrent first-trimester intrahepatic cholestasis of pregnancy. J Perinatol 2014;34:711—2.
[102] Elderman JH, ter Borg PCJ, Dees J, Dees A. Pregnancy and *ABCB4* gene mutation: risk of recurrent cholelithiasis. BMJ Case Rep 2015:2015, http://dx.doi.org/10.1136/bcr-2014-206919.
[103] Degiorgio D, Crosignani A, Colombo C, Bordo D, Zuin M, Vassallo E, et al. ABCB4 mutations in adult populations with cholestatic liver disease: impact and phenotypic expression. J Gastroenetrol 2015, http://dx.doi.org/10.1007/s00535-015-1110-z [E-pub ahead of print].

[104] Eloranta ML, Hakli T, Hiltunen M, Helisalmi S, Punnonen K, Heinonen S. Association of single nucleotide polymorphisms of the bile salt export pump gene with intrahepatic cholestasis of pregnancy. Scand J Gastroenterol 2003;38:648—52.
[105] Painter JN, Savander M, Sistonen P, Lehesjoki AE, Aittomaki K. A known polymorphism in the bile salt export pump gene is not a risk allele for intrahepatic cholestasis of pregnancy. Scand J Gastroenterol 2004;39:694—5.
[106] Davit-Spraul A, Gonzales E, Jacquemin E. NR1H4 analysis in patients with progressive familial intrahepatic cholestasis, drug-induced cholestasis or intrahepatic cholestasis of pregnancy unrelated to ATP8B1, ABCB11 and ABCB4 mutations. Clin Res Hepatol Gastroenterol 2012;36:569—73.
[107] Owen BM, Van Mil SW, Boudjelal M, McLay I, Cairns W, Elias E, et al. Sequencing and functional assessment of hPXR (NR1I2) variants in intrahepatic cholestasis of pregnancy. Xenobiotica 2008;38:1289—97.
[108] Castano G, Burgueno A, Fernandez Gianotti T, Pirola CJ, Sookoian S. The influence of common gene variants of the xenobiotic receptor (PXR) in genetic susceptibility to intrahepatic cholestasis of pregnancy. Aliment Pharmacol Ther 2010;31:583—92.
[109] Savander M, Ropponen A, Avela K, Weerasekera N, Cormand B, Hirvioja ML, et al. Genetic evidence of heterogeneity in intrahepatic cholestasis of pregnancy. Gut 2003;52:1025—9.
[110] Keitel V, Vogt C, Haussinger D, Kubitz R. Combined mutations of canalicular transporter proteins cause severe intrahepatic cholestasis of pregnancy. Gastroenterology 2006;131:624—9.
[111] Wasmuth HE, Glantz A, Keppeler H, Simon E, Bartz C, Rath W, et al. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter *ABCB4* gene. Gut 2007;56:265—70.
[112] Meier Y, Zodan T, Lang C, Zimmermann R, Kullak-Ublick GA, Meier PJ, et al. Increased susceptibility for intrahepatic cholestasis of pregnancy and contraceptive-induced cholestasis in carriers of the 1331T>C polymorphism in the bile salt export pump. World J Gastroenterol 2008;14:38—45.
[113] Muehlenberg K, Wiedmann K, Keppeler H, Sauerbruch T, Lammert F. [Recurrent intrahepatic cholestasis of pregnancy and chain-like choledocholithiasis in a female patient with stop codon in the *ABDC4*-gene of the hepatobiliary phospholipid transporter]. Z Gastroenterol 2008;46:48—53.
[114] Favre N, Abergel A, Blanc P, Sapin V, Roszyk L, Gallot D. Unusual presentation of severe intrahepatic cholestasis of pregnancy leading to fetal death. Obstet Gynecol 2009;114:491—3.
[115] Zimmer V, Mullenbach R, Simon E, Bartz C, Matern S, Lammert F. Combined functional variants of hepatobiliary transporters and FXR aggravate intrahepatic cholestasis of pregnancy. Liver Int 2009;29:1286—8.
[116] Zimmer V, Krawczyk M, Mahler M, Weber SN, Mullenbach R, Lammert F. Severe hepatocellular dysfunction in obstetric cholestasis related to combined genetic variation in hepatobiliary transporters. Clin Exp Obstet Gynecol 2012;39:32—5.
[117] Mella JG, Roschmann E, Glasinovic JC, Alvarado A, Scrivanti M, Volk BA. Exploring the genetic role of the HLA-DPB1 locus in Chileans with intrahepatic cholestasis of pregnancy. J Hepatol 1996;24:320—3.
[118] Eloranta ML, Heiskanen J, Hiltunen M, Helisalmi S, Mannermaa A, Heinonen S. Apolipoprotein E alleles in women with intrahepatic cholestasis of pregnancy. Scand J Gastroenterol 2000;35:966—8.
[119] Chen F, Fan L, Xu L, Yao F. [Relationship between human leukocyte antigen-DRB1 allele gene polymorphism and intrahepatic cholestasis of pregnancy]. Zhonghua Fu Chan Ke Za Zhi 2002;37:519—22.
[120] Heinonen S, Eloranta ML, Heiskanen J, Punnonen K, Helisalmi S, Mannermaa A, et al. Maternal susceptibility locus for obstetric cholestasis maps to chromosome region 2p13 in Finnish patients. Scand J Gastroenterol 2001;36:766—70.
[121] Laasanen J, Hiltunen M, Romppanen EL, Punnonen K, Mannermaa A, Heinonen S. Microsatellite marker association at chromosome region 2p13 in Finnish patients with preeclampsia and obstetric cholestasis suggests a common risk locus. Eur J Hum Genet 2003;11:232—6.
[122] Zhang L, Liu S, Chen Q, Yang X, Chen X. [Human leucocyte antigen-DPA1 polymorphism distribution in Chengdu Chinese families with intrahepatic cholestasis of pregnancy]. Sichuan Da Xue Xue Bao Yi Xue Ban 2003;34:530—2.
[123] Zhang L, Liu SY, Shi QY, Chen Q, Zou H, Xing AY. [Study on the association between estrogen receptor alpha gene polymorphism and intrahepatic cholestasis of pregnancy]. Zhonghua Fu Chan Ke Za Zhi 2006;41:307—10.
[124] Zhang L, Liu SY, Chen Q, Zou H, Zuo YQ, Xu XH, et al. [An associated analysis of estrogen receptor 2 gene polymorphism linked with intrahepatic cholestasis of pregnancy]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;23:434—6.
[125] Zhang L, Liu SY, Chen Q, Shi QY, Zou H, Wu L. [Association between polymorphisms of CYP17 and CYP3A4 genes and intrahepatic cholestasis of pregnancy in Chengdu]. Sichuan Da Xue Xue Bao Yi Xue Ban 2006;37, 551—3, 82.
[126] Peng B, Chen Q, Zhang L, Zou H, Liu SY. [Association of genetic polymorphisms in human leukocyte antigen-DQA1 with intrahepatic cholestasis of pregnancy in Chengdu district]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;23:555—7.
[127] Peng B, Liu SY, Chen Q, Wang XD, Zhang L, Zou H. [Expression of human leucocyte antigen G on human placenta and its gene polymorphism in relation to intrahepatic cholestasis of pregnancy]. Zhonghua Fu Chan Ke Za Zhi 2007;42:443—7.
[128] Laasanen J, Helisalmi S, Iivonen S, Eloranta ML, Hiltunen M, Heinonen S. Gamma 2 actin gene (enteric type) polymorphism is not associated with obstetric cholestasis or preeclampsia. Fetal Diagn Ther 2008;23:36—40.
[129] Wang X, Zhang L, Ou R, Wang J, Liu S, Chen Q, et al. [Association between *CYP1A1* gene polymorphism and intrahepatic cholestasis of pregnancy]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2008;25:70—2.
[130] Wang X-L, Tan X, Zhang L, Ou R-G, Yan A-H, Chen Q, et al. [Association of gene polymorphisms of CYP1B1 with intrahepatic cholestasis of pregnancy]. Sichuan Da Xue Xue Bao Yi Xue Ban 2008;39:434—7.
[131] Yin Y, Zhu Q-Y, Ren S-J, Wang D-M. [Association between estrogen receptor beta gene polymorphism and intrahepatic cholestasis of pregnancy in the Uygurs and the Hans pregnant women]. Zhonghua Yu Fang Yi Xue Za Zhi 2012;46:269—72.
[132] Germanova A, Muravska A, Jachymova M, Hajek Z, Koucky M, Mestek O, et al. Receptor for advanced glycation end products (RAGE) and glyoxalase I gene polymorphisms in pathological pregnancy. Clin Biochem 2012;45:1409—14.