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REVIEW ARTICLE

Intrahepatic cholestasis of pregnancy: insights into pathogenesis and advances in omics studies

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Abstract

Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-specific liver disease. It is characterized by pruritus, abnormal liver function and elevated total bile acid (TBA) levels, increasing the risk of maternal and fetal adverse outcomes. Its etiology remains poorly elucidated. Over the years, various omics techniques, including metabolomics, microbiome, genomics, etc., have emerged with the advancement of bioinformatics, providing a new direction for exploring the pathogenesis, diagnosis and treatment of ICP. In this review, we first summarize the role of bile acids and related components in the pathogenesis of ICP and then further illustrate the results of omics studies.

Keywords Intrahepatic cholestasis of pregnancy · Bile acid · Bile acid receptor · Metabolomics · Microbiome · Genomics · Lipid · Hormone · Gut microbiota · Gene

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is the most common pregnancy-specific liver disease, with an estimated incidence ranging from 0.3% to 15% in various populations [1]. ICP is characterized by mild to severe pruritus, abnormal liver function and elevated total bile acid (TBA) levels in the third trimester of pregnancy. The severity of ICP is graded according to the TBA concentration: mild ICP, 10–39 μmol/L, and severe ICP, TBA ≥ 40 μmol/L. Although this disease is generally mild in pregnant women and recovers after delivery, it increases the risk of postpartum hemorrhage, susceptibility to hepatobiliary cancer, immune diseases and cardiovascular diseases [2]. Moreover, ICP can be highly harmful to the fetus, as maternal BAs can pass through the placenta and accumulate in the fetus and amniotic fluid, leading to various complications, as described below.

Patients with ICP have a higher-than-normal stillbirth rate, approximately 0.1–0.3% from 37 weeks of gestation [3]. The highest risk for stillbirth is in women with TBA ≥ 100 μmol/L [4], and the incidence of stillbirth in twin pregnancies is significantly higher than that in singleton pregnancies [5]. The pathophysiology of stillbirth may be related to BAs, which cause an acute fetal anoxic event possibly due to acute placental vessel spasm [6] or fetal arrhythmia [7]. The degree of fetal cardiac dysfunction

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is also closely linked to the level of serum TBA [7], and fetal myocardial deformation is more likely to be impaired in severe ICP mothers (TBA ≥ 40 μmol/L) [8]. The risks of preterm delivery, meconium-stained amniotic fluid (MSAF), RDS, and stillbirth also rise with the elevation of maternal TBA to ≥ 40 μmol/L [9–11] (Table 1).

Although a meta-analysis of more than 5000 women with ICP discovered that ICP is not associated with low birth weight (LBW), with no difference in birthweight percentile between the ICP and control groups, a retrospective cohort study of 68,245 singleton pregnancies revealed LBW and a higher incidence of intrauterine growth restriction (IUGR) (1.4% vs. 0.5%) in patients with serum TBA ≥ 4.08 μg/mL, approximately 10 μmol/L [12]. Patients with the symptom of steatorrhea may have malabsorption of vitamin K in ICP [13]. Steatorrhea and vitamin K deficiency can lead to postpartum hemorrhage [14].

The pathogenesis of ICP is associated with genetic, hormonal and immunological factors, but the etiology of the disease is still poorly elucidated. In this review, we first summarize the role of bile acids and related components in the pathogenesis of ICP and then go into the omics results (metabolomics, microbiome, genomics, etc.) to further explore the pathogenesis of ICP.

BA regulation in ICP and other liver diseases

In liver diseases, cholestasis related to BA regulation can be divided into intrahepatic cholestasis (IHC) and extrahepatic cholestasis (EHC) according to the presence of obstruction. EHC is caused by obstruction of excreta outside the liver and extrahepatic bile duct disease. In contrast, hepatic parenchymal cell and/or intrahepatic bile duct diseases contribute to IHC. These include primary sclerosing cholangitis (PSC), which is found in IHC more than any other condition, progressive familial intrahepatic cholestasis (PFIC), and ICP [15].

According to the location and mechanism of cytological damage, PSC, PFIC and ICP are associated with a reduction of bile canaliculi, genetic defects in bile transporters, and changes in the canalicular membrane of bile canaliculi, respectively, finally resulting in defects in BA synthesis and abnormal bile excretion. The etiology of PSC is still unclear. PFIC is an autosomal recessive disease, while ICP is due to various factors, environmental, hormonal and genetic.

ICP is characterized by pruritus and elevated serum BAs, which are necessary for the diagnosis of ICP. However, BA elevation may not be apparent in other, nonpregnant IHC patients, so serum BAs are not diagnostic criteria for other IHCs.

Enterohepatic circulation of bile acids

In humans, the primary BAs synthesized include chenodeoxycholic acid (CDCA) and cholic acid (CA), which are regulated by cholesterol 7a-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1). In addition, CA production is regulated by sterol 12a-hydroxylase (CYP8B1), which can affect the ratio of these two primary BAs [16]. After CDCA and CA bind to taurine or glycine at a ratio of approximately 1:3 [17], these conjugated BAs are delivered into the bile duct via the bile salt export pump (BSEP), which is a specific ATP-dependent transporter encoded by the ABCB11 gene. Mutations in the ABCB11 gene are thought to increase susceptibility to ICP [18]. Phospholipids in bile require multidrug resistance protein 3 (MDR3), encoded by the ABCB4 gene. Mutations in the ABCB4 gene have a major role in the pathogenesis of ICP [19], and these mutations are related to the severity of ICP and to TBA levels above 40 μmol/L [20]. Cholesterol is transported via ATP-binding cassette transporter G5 (ABCG5)/ATP-binding cassette transporter G8 (ABCG8). Multidrug resistance-associated transporter 2 (MRP2), expressed in the canalicular membrane of hepatocytes, is encoded by the ABCC2 gene and mainly secretes conjugated bilirubin, glutathione conjugates and other organic anion compounds. MRP2 also contributes to the development of ICP, although this relationship was found only in a population of South American women and not Caucasians [21].

Then, conjugated BAs stored in the gallbladder can be released into the duodenum under the influence of postprandial cholecystokinin. Only a small fraction of BAs diffuses passively in the duodenum. Conjugated BAs are reabsorbed in the distal ileum by apical sodium-dependent transporter

Table 1 Relationship of bile acid and ICP

ICP grade	BA concentrations	Complications closely related to BA concentrations	Delivery time (Recommended by SMFM)
Mild ICP	10–39 μmol/L	LBW; IUGR; Fetal cardiac dysfunction	$36^{0/7}$–$39^{0/7}$ weeks of gestation
Severe ICP	≥ 40 μmol/L	Preterm delivery; MSAF; RDS; Fetal myocardial deformation	$36^{0/7}$–$39^{0/7}$ weeks of gestation
	≥ 100 μmol/L	Stillbirth	$36^{0/7}$ weeks of gestation

LBW low birth weight, *IUGR* intrauterine growth restriction, *SMFM* society for maternal fetal medicine, *MSAF* meconium-stained amniotic fluid, *RDS* respiratory distress syndrome

(ASBT). Then, BAs can efflux into the portal blood by heterodimeric transporter organic solute transporters alpha/beta (OSTα/OSTβ), and this progress can be facilitated by intestinal bile acid-binding protein (IBABP). Na+/taurocholate cotransporter (NTCP) and organic anion transporting polypeptides (OATPs), respectively, mediate the uptake of bile salts and bile acids from the blood to the liver [22]. This process is termed enterohepatic circulation. NTCP performs the majority of BA uptake, and its expression is affected by BAs and hormones, such as estrogen and prolactin [22]. Hepatic multidrug resistance protein 3 (MRP3), multidrug resistance protein 4 (MRP4), and OSTα/OSTβ, which exist at the hepatic basolateral membrane as well as in enterocytes, provide excretion routes for BAs into the circulation. MRP3 and MRP4 have low expression in the physiological state but are upregulated in ICP [17].

In addition, BAs escaping ileal reabsorption can be exposed to intestinal flora. Microbial deconjugation, as in bacteria with bile salt hydrolase (BSH) activity, can remove glycine or taurine from conjugated BAs, preventing active reabsorption via ASBT, and these BAs finally enter the colon to become secondary BAs [deoxycholic acid (DCA) and lithocholic acid (LCA)] by 7-dehydroxylation. Approximately 95% of BAs are taken up by the liver through enterohepatic circulation, and 5% of BAs are excreted in the feces [23] (Fig. 1).

In addition to the effects of the ABCB11, ABCB4, and MRP2 genes mentioned above, mutations in the ATP8B1 gene, encoding familial intrahepatic cholestasis protein 1 (FIC1), which are often identified in PFIC, are also found in ICP [24]. FIC1 was proposed to be an amino-phospholipid translocase that may act to maintain the distribution of phospholipids and thus the function of BA transporters [24].

Beyond the influence of genetic defects in enterohepatic circulation, hormones, such as estrogen and progesterone, which are at their highest levels in the late stages of pregnancy, when ICP generally occurs, can also inhibit BA flow through the liver. 17β-Estradiol can repress BSEP expression, affecting biliary secretion of BAs [25]. In rat studies, one estrogen–glucuronide, estradiol-17β-D-glucuronide, trans-inhibits the bile suit export pump (BSEP) [26]. Estradiol-17β-D-glucuronide is excreted into bile by MRP2 in drug- and estrogen-induced cholestasis [27] and suppresses the expression of MRP2 in intrahepatic and obstructive cholestasis [28]. Like estrogen, one sulfated progesterone, allopregnanolone sulfate (PM4S), can cause trans-inhibition of BSEP [26] in ICP. The sulfated progesterone PM4-S/epiallopregnanolone–sulfate (PM5S) competitively inhibits the NTCP-mediated uptake of taurocholate (TC) [29].

Pregnancy can bring about portal hypertension syndrome due to modified systemic hemodynamics as a response to the increased oxygen demands of the fetus and mother. Despite the paucity of associations between portal hypertension and ICP in previous studies, as increased plasma volume is related to increased aldosterone, estrogen, and lactogen levels, ICP has also become a risk factor for preeclampsia [30].

Overall, in the pathophysiological process of ICP, the synthesis of BAs can increase under the influence of CYP7A1 and CYP8B1, while the transport of BAs is impaired due to effects on BA transporters such as BSEP and MDR3. In addition, the BA profile is also altered. Although CA and CDCA are the predominant serum BAs in ICP patients, the level of CA is significantly higher in ICP patients than in normal pregnant women, while CDCA is modestly increased or even a decreased, thereby further increasing the CA/CDCA ratio [31]. There is also a rise in the concentrations of secondary BAs. These profile changes, including pool size, may indicate alterations in the hydrophilicity and hydrophobicity of the BA pool. Hydrophobic BAs are typically more toxic and can damage cell membranes and promote oxidative stress, apoptosis and necrosis [32].

Placental bile acids

In normal pregnant women, serum bile acid levels may increase gradually with advancing gestation [33–35]. Maternal BA synthetic pathways are present not only in the liver in the enterohepatic circulation but also in the placenta [36]. On the other hand, the fetus has the ability to synthesize BAs as early as 12 weeks of gestation [37], though its enterohepatic circulation is not yet functional. BAs produced by the fetus are mainly processed by the placenta, and a small fraction is excreted to amniotic fluid via the fetal kidneys. In human amniotic fluid, CA and CDCA were found in all test samples, while only a portion detected LCA and DCA [38]. CA and CDCA increased significantly in the amniotic fluid of ICP patients despite there being no change in the concentration of DCA [39].

Various enzymes and transports of BAs assist in maintaining BA homeostasis. The sulfation of BAs, activated by placental sulfatases (SULFs) and sulfotransferases (SULTs), and the glucuronidation of BAs, catalyzed by UDP glucuronosyltransferases (UGTs), lead to the production of water-soluble BAs and facilitate excretion [40, 41]. These processes form a metabolic barrier that avoids the transfer of potentially toxic hydrophobic BAs from the maternal to fetal circulation. Transport of BAs also plays an active role in protecting the fetus from BA overexposure. Trophoblast cells in the placenta have a maternal-facing apical membrane and a fetal-facing basolateral membrane. [17] The OATP family can transport fetal BAs across the basal plasma membrane, while NTCP is poorly expressed in the placenta and is used to mediate bile acid uptake in the liver. [42] Other transporters, including MDR3, OSTα, organic cation transporter

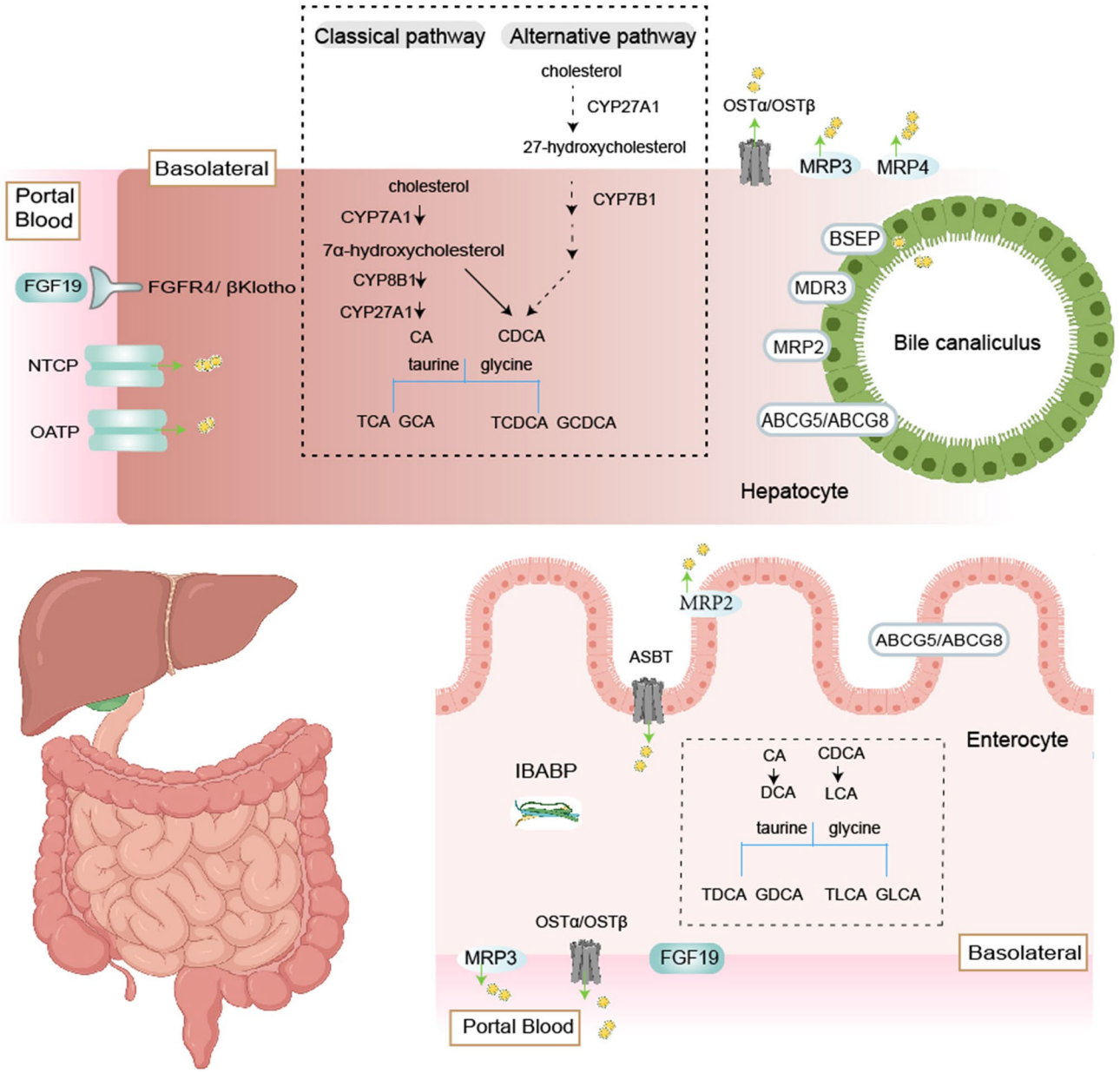

Fig.1 Enterohepatic circulation of bile acides and the main bile acid transporters in enterohepatic system. The classical and alternative pathway of primamry BA synthesis are indicated by solid line and dotted line respectively, generating taurine- or glycine- conjugated BAs such as TCA, GCA, TCDCA and GCDCA. Active transporters BSEP, MDR3, ABCG5/ABCG8 and MRP2 deliver conjucated BAs phospholipids, cholesterol and bilirubin into the bile duct, respectively. Then, conjugated BAs are reabsorbed in the distal ileum by ASBT. Most BAs are transported from the intestine back to the liver via transporters on ileal enterocytes (OSTα/OSTβ) and hepatocytes (NTCP and OATP). FGF19 from the enterocytes can bind tok FGFR4/β-Klotho on the hepatocytes, leading to suppression of BA synthesis. *CA* cholic acid, *CDCA* chenodeoxycholic acid, *GCA* glycholic acid, *TCA* taurocholic acid, *GCDCA* glycochenodeoxycholic acid, *TCDCA* taurochenodeoxycholic acid, *DCA* deoxycholic acid, *LCA* lithocholic acid, *GDCA* glycodeoxycholic acid, *TDCA* taurodeoxycholic acid, *TLCA* taurolithocholoc acid, *GLCA* glylithocholic acid. Figure created with BioRender.com

3 (OCT3), and organic anion transporter 4 (OAT4), are also expressed at the basal membrane. MDR1, MRP2, and ABCG2 have been found at the apical membrane. ABCG2, which is also located in fetal vessels of the chorionic villi, plays a key role in transporting hepatobiliary products such as sulfated and nonsulfated BAs across the placenta. MRP1 and MRP3, which are expressed in the endothelium of fetal blood vessels, are less abundant in the apical membrane, and BSEP is expressed at low levels in the placenta [17] (Fig. 2).

When ICP occurs, the interaction of BAs at the level of the feto-maternal axis in the placenta is altered, and BA flow is reversed from mother to fetus due to the high maternal BA

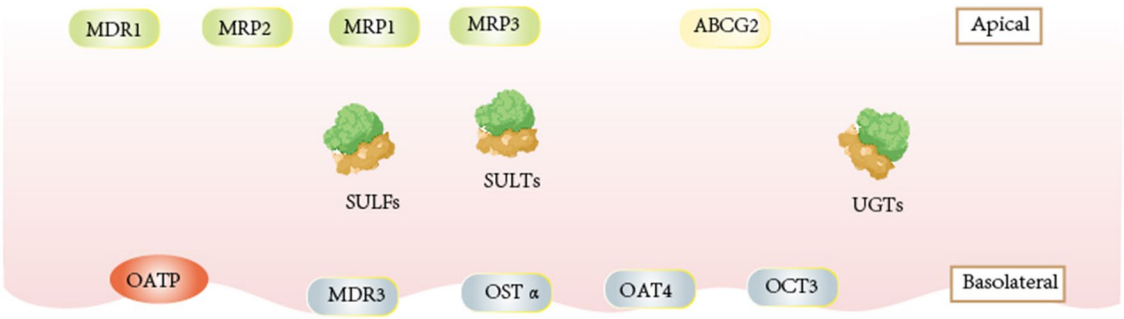

Fig. 2 Bile acid transporters and enzymes in the placenta. Placental SULFs and SULTs convert unconjugated steroids such as BAs and estrogens, into their sulfated forms; UGTs catalyse the glucuronidation of BAs. *SULTs* sulfotransferases, *SULFs* sulfatases, *UGTs*, *UDP* glucuronosyltransferases. Figure created with BioRender.com

levels. High concentrations of BAs, especially hydrophobic BAs such as LCA, may exert a detrimental effect on the fetus by constricting chorionic veins [6, 43]. Placental transport of BAs was affected and the RNA expression of OATP3A1 was significantly decreased in ICP patients [44]. Placental MRP2 protein and RNA expression soared in ICP patients treated with UCDA compared to controls; meanwhile, MRP3 protein expression was not obviously different, while the RNA expression was significantly decreased [45].

Bile acid receptors

BAs can influence multiple metabolic pathways in many tissues primarily by activating nuclear receptor farnesoid X receptor (FXR) and G protein-coupled receptor (TGR5) [16].

FXR is widely distributed in a variety of tissues and is predominantly expressed in the liver, intestine and kidneys. In hepatocytes, primary BAs can bind to FXR and activate the FXR–RXR heterodimer complex, which can enhance the expression of small heterodimer protein (SHP). SHP binds to liver receptor homolog-1 (LRH-1), repressing CYP7A1. SHP can inhibit the expression of NTCP. Activation of FXR can promote BSEP, MDR3, and MPR2, inducing efflux of bile into the bile canaliculus. In enterocytes, FXR activated by BAs can induce the expression of fibroblast growth factor 19 (FGF19), SHP, OSTα/OSTβ and IBABP. FGF19 is secreted into enterohepatic circulation and binds to a complex of the receptor tyrosine kinase FGF receptor 4 (FGFR4)/β–Klotho on hepatocytes, resulting in the initiation of JNK/ERK signaling, which can blunt the expression of CYP7A1. Cyp8b1 expression can also be downregulated by the activation of FXR, modulating CA production [46]. On the other hand, upregulation of SHP by FXR may inhibit ASBT in animal model, reducing the uptake of intestinal BAs. [47].

TGR5 is a plasma membrane-bound G protein widely expressed in the gallbladder, placenta, intestine, lung, spleen, brown and white adipose tissue, etc. In the placenta, it is found mainly in fetal macrophages and to a lesser extent in trophoblasts [48]. TGR5 is predominantly activated by secondary BAs, and its affinity is as follows: LCA > DCA > CDCA > CA. BAs conjugated to taurine are generally more potent activators than BAs conjugated to glycine or unconjugated BAs [23]. TGR5 located on enteroendocrine L cells can mediate BA-induced cyclic AMP (cAMP) increases and protein kinase A (PKA) activation. Activation of TGR5 also increases the synthesis and release of GLP-1 and modulates calcium mobilization. Furthermore, although FXR can stimulate TGR5 expression, activation of FXR in L cells inhibits GLP-1 synthesis (Fig. 3).

Influence of bile acid metabolism

BA biosynthesis can terminate with the conjugation of glycine or taurine in humans. Additional forms of BA conjugation include sulfation, hydroxylation and glucuronidation [23].

An untargeted metabolomics study [49] showed that glycochenodeoxycholate (GCDCA), taurochenodeoxycholate (TCDC), glycocholic acid (GCA), TC, glycolithocholic acid (GLCA) and taurodeoxycholic acid (TDCA) in plasma were increased in the ICP group. Furthermore, taurine negatively interacted with GCDCA, TCDC and GCA, according to debiased sparse partial correlation (DSPC) networks. Taurine, which is an important free radical scavenger and an antioxidant, was also found to be significantly decreased in ICP. Taurine may attenuate maternal oxidative stress or improve adverse pregnancy outcomes in rats [50, 51].

Another study [52] enrolled ICP patients with gestational age < 28 weeks as the early onset ICP (EICP) group and ICP patients with gestational age ≥ 28 weeks as the late-onset ICP (LICP) group. Conjugated BAs increased in the

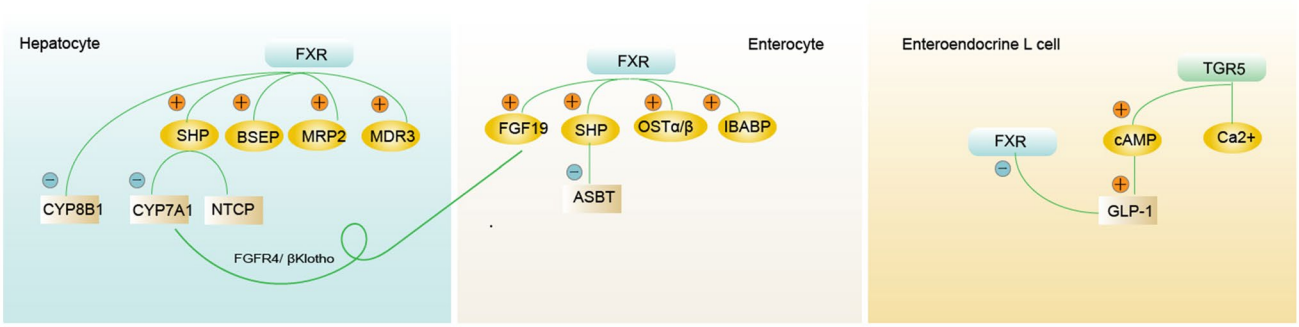

Fig. 3 Function mechanism of bile acid receptors FXR and TGR5 in hepatocyte, entercyte and enteroendocrine L cells

serum of ICP patients, whereas GCA was especially highly expressed in the EICP group, and taurocholic acid (TCA) was expressed in the LICP group, showing distinct BA metabolism profiles of EICP and LICP.

In a study that collected serum, placenta and urine samples [53], three main pathways of primary BA biosynthesis, taurine metabolism, hypotaurine metabolism, and sphingolipid metabolism, were found in serum samples, while only the main pathway of primary BA biosynthesis was found in placenta and urine. GCDCA, taurochenodesoxycholic acid (TCDCA), GCA and TCA increased in all samples of ICP patients. This systematic metabolomics profiling showed that the placental and serum states tended to be very similar, whereas the urine state was distinct.

Above all, the importance of glycine and taurine conjugation was demonstrated. Sulfation also plays a crucial role in the detoxification of BAs, increasing their solubility, decreasing their intestinal absorption, and promoting urinary and fecal excretion [54]. In humans, in contrast to the small fraction of BAs in bile and serum being sulfated, more than 70% of BAs can be sulfated in urine. The hydroxysteroid SULT family is composed of two subfamilies, SULT2A1 and SULT2B1. SULT2A1 has a limited distribution in tissue, whereas SULT2B1 is detected in various hormone-responsive tissues, such as the uterus, ovary, and placenta [55]. In the placenta, SULT2B1 is localized to the nuclei of placental syncytiotrophoblasts [41] (Fig. 2).

In a targeted metabolomics study of sulfated bile acids (SBAs) in urine [56], total SBAs were found to be increased in ICP, especially sulfated taurine-amidated BAs (TBA-S) and glycine-amidated BAs (GBA-S). For example, sulfated dihydroxy glycine bile acid (di-GBA-S), sulfated dihydroxy taurine bile acid (di-TBA-S), glycine cholic acid 3-sulfate (GCA-3S) and taurine cholic acid 3-sulfate (TCA-3S) increased significantly in the ICP group. When BAs were conjugated to glycine and taurine, this decreased their pKa, which increased their solubility and enhanced their urinary elimination. GBA-S was the major SBA in urine, whereas the uptrend in the proportion of TBA-S had a higher correlation with the severity of ICP than that of GBA-S. TBA-S is generally less cytotoxic than GBA-S. In that study, GCA-3S was well suited as a biomarker for the diagnosis of ICP, and the combination of GCA-3S and di-GBA-S-1, which were constructed by multivariable logistic regression, was suitable for the grading of ICP. In addition, sulfated unconjugated BAs, such as LCA-3S, remained at low concentrations.

In another untargeted metabolomics study of urine [57], most of the detected metabolites were involved in BA biosynthesis and metabolism, hormone metabolism and lipid metabolism. Varanic acid, tauromuricholic acid (TMCA), GCA, chenodeoxycholic acid 3-sulfate (CDCA-3S), glycochenodeoxycholate-3-sulfate (GCDCA-3S), and taurohyocholate (THC) were found to be increased in the ICP group. A metabolite panel [L-homocysteine sulfonic acid, GCA and CDCA-3S, MG (22:5), LysoPE (22:5)] was screened out based on binary logistic regression analysis, having high diagnostic accuracy for ICP.

Furthermore, hydroxylation and glucuronidation of BAs occupy an important position in ICP, which are conducive to solubility and excretion of BAs. The common BAs in humans can be sorted by hydrophobic strength as follows: muricholic acid (MCA) < ursodeoxycholic acid (UDCA) < CA < CDCA < DCA < LCA [16]. LCA is a toxic BA that can induce endoplasmic reticulum stress and syncytiotrophoblast cell apoptosis in ICP patients [58, 59]. In a targeted metabolomics study of plasma BAs[60], hydroxylated BAs such as UDCA, hyocholic acid (HCA) and MCA, which are converted from CDCA, were increased in the ICP group. HCA and MCA were the predominant BAs in pigs and rodents, respectively, but showed low concentrations in humans [61, 62]. CDCA and LCA remained unchanged between the ICP and normal groups. Glucuronidated BAs such as CDCA-3Gln and sulfated BAs such as LCA-3S, GLCA-3S, and TLCA-3S were significantly increased in severe ICP patients [60]. In this study, taurine-conjugated BAs, glycine-conjugated BAs, and CA/CDCA increased in the ICP groups. In contrast to

the higher levels of glycine-conjugated BAs in the healthy group, taurine-conjugated BAs increased more in the ICP group, to be slightly more abundant than glycine-conjugated BAs. THCA, GHCA, GLCA and TLCA-3S may be biomarkers for ICP grading.

In a pseudotargeted metabolomics study of serum BAs in women with ICP [63], the BA profile was altered due to the reduced proportion of unconjugated BAs and increased proportion of taurine and glycine conjugates in women with ICP, especially TCA, GCA, TCDC, T-ω-MCA, THCA, and Ttri-3, which soared in the ICP group. After delivery, these conjugates decreased and unconjugated BAs increased. In addition, the proportion of unconjugated BAs increased significantly during UDCA therapy. A potential combination biomarker made up of α-MCA, TCA, and Gtri-8 had good predictive ability by logistic regression analysis. α-MCA was first discovered in Murinae and was discovered in human serum by advanced detection techniques. In another combination study detecting placental tissue through metabolomics and proteomics [64], GCA was also increased in the ICP group and was found to be a potential biomarker with acyl-CoA oxidase 1 (ACOX1) and L-palmitoylcarnitine.

Noteworthy changes in BAs in the placenta, urine and blood of ICP patients according to metabolomics studies are illustrated in Fig. 4.

Influence of lipid metabolism

The chemical structure of BAs gives them detergent-like activity; once released postprandially in the duodenum, the BA fraction facilitates lipid emulsification and absorption. Hence, lipid metabolism shows a significant change in the setting of ICP. Lipids play important roles in various biological processes, including intercellular communication, energy transport and signal transduction.

Sphingosine 1-phosphate (S1-P), a bioactive sphingolipid, phosphatidylcholines (PCs) and Lysophosphatidylcholine (LysoPC) in plasma were found to be reduced in an ICP cohort [49]. LysoPCs increased in the placenta of ICP patients in another study, which also portrayed reduced diacylglycerol and increased sphingoid bases in the serum of ICP patients [53]. This study found that sphingolipid metabolism was important in the metabolic pathway of ICP [53]. Another untargeted lipidomics study [65] confirmed this result, indicating a connection between ICP and disordered sphingolipid homeostasis, especially ceramide (Cer) and sphingomyelin (SM). In these plasma lipid profiles [65], 33 lipids differentially expressed in ICP group compared with the control group, and 20 sphingolipids accounted for most of this difference. All differentially abundant sphingolipids in the mild ICP group were also differentially abundant in the severe ICP group, and the trend of expression was positively correlated with disease severity. Sphingolipids are

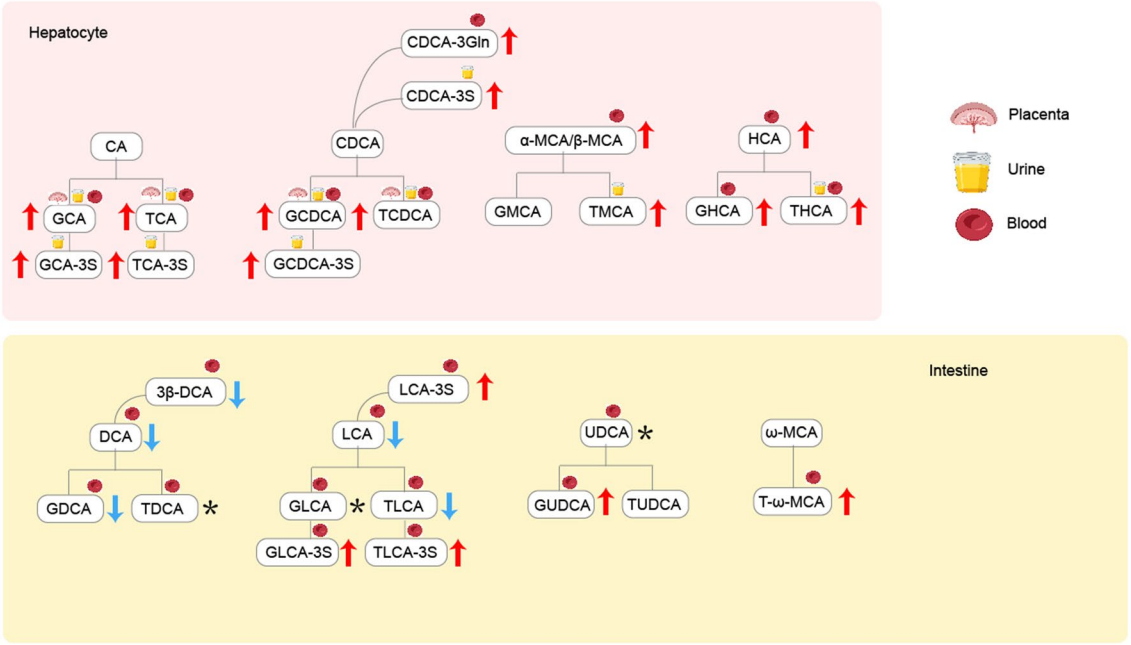

Fig. 4 Remarkable changes of bile acids in placenta, urine and blood of ICP patients according to metabolomics studies. Note: Red Arrow means BAs increases in ICP; Blue arrows means BAs decrease in ICP; Asterisk means BAs increase in plasma while decreased in serum. Figure created with BioRender.com

ubiquitous structural components present in eukaryotic cell membranes that modulate a wide range of biological processes, including cell proliferation, immune cell trafficking and inflammation. Sphingolipids are also the main ligands for the G protein-coupled receptor sphingosine 1-phosphate receptor 2 (S1PR2) [66], which interacts with BAs to trigger acute responses. S1PR2 may play a pathological role in cholestasis, as the blockage of S1PR2 attenuated portal vein pressure and liver injury in rodents [67, 68].

Thus, lipids, especially sphingolipids, are important in the occurrence of ICP. However, the above metabolomics studies only reflect the changes in lipids, and the potential mechanisms of lipid functions in ICP remain obscure. Bile acid-mediated activation of S1PR2 and FXR may play key roles, as BAs may be associated with lipid metabolism through activation of S1PR2 [66], and FXR regulates lipid metabolism in a rodent model [69], while downregulation of FXR activity is involved in ICP pregnancies [70]. Further studies are needed.

Influence of hormones

It is widely known that estrogen and progesterone play key roles in the pathogenesis of ICP. As sulfation and glucuronidation are primary metabolic pathways of hormones, estrone glucuronide and estriol-3-glucuronide in urine were found to be increased in ICP patients [57]. However, pregnanolone sulfate, pregnandiol sulfate, 16a-hydroxy DHEA 3-sulfate, pregnanediol monosulfate, 5β-pregnanediol sulfate, androsterone sulfate, and testosterone sulfate were found to be decreased in ICP [53].

In another study collecting plasma samples, estriol 16-glucuronide, estrone glucuronide, and estrone sulfate in plasma were also found to soar in ICP [49]. Estrone sulfate, the most abundant circulating estrogen in pregnant women, acts as a long-term storage that can be transformed to more active estradiol as needed. It was found to inhibit TC uptake by 65% but did not decrease bile flow following administration to rats, under conditions in which estradiol-17β-D-glucuronide decreased bile flow by 100% [71].

Unlike its decrease in urine [53], pregnenolone sulfate was found to be increased significantly in plasma [49]. Progesterone plays a key role in the pathophysiology of ICP, and pregnenolone sulfate is the precursor of progesterone and is converted into progesterone by enzymes. Sulfated progesterone metabolites were reported to increase BA levels by inhibiting FXR, thus reducing FXR-mediated bile acid efflux and secreted FGF19 [70].

Overall, these metabolomics studies discovered some important hormones acting in ICP, such as estrone glucuronide, estrone sulfate, and pregnanolone sulfate, which influence the uptake and transport of BAs, though their detailed mechanisms in the occurrence of ICP require further investigation.

Role of gut microbiota

Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria are generally known as the dominant phyla in pregnant women [72]. During ICP, the relative abundance of Firmicutes was reduced, and Bacteroidetes was enriched [73, 74]. The genome of bacteria of the Bacteroidetes phylum can encode BSH capacity [75]. UDCA treatment of ICP patients has been associated with enrichment of the gut microbiota with Bacteroidetes; as a result, the increased BSH activity deconjugated BAs, enabling secondary modification to FXR agonists, increasing FGF19-mediated enterohepatic feedback and reducing 7α-hydroxy-4-cholesten-3-one (C4) concentrations [74].

A microbiome study in ICP revealed that at the genus level, Blautia, Citrobacter and Streptococcus were significantly higher in the ICP group, while at the family level, Enterobacteriaceae, Leuconostocaceae and Streptococcaceae increased in the ICP group. In KEGG pathways, differences in ketogluconate metabolism were more pronounced in ICP patients [76].

In another study that combined gut microbiota with the serum metabolome [73], at the genus level, bacteria depleted in ICP contained butyrate-producing bacteria, such as Faecalibacterium, Eubacterium hallii and Blautia, which were capable of producing short-chain fatty acids, and Bifidobacterium, which exhibited functional BSH [77], preventing the reuptake of BAs from the small intestine. In contrast to the findings mentioned above, Blautia, which has beneficial roles in glucose metabolism [78, 79], was reduced in ICP. Blautia was also found to be elevated in primary sclerosing cholangitis when bile release into the small intestine was inhibited [80]. Furthermore, the bacteria enriched in ICP were involved in BA metabolism, such as Parabacteroides and Bilophila. Escherichia/Shigella, which may promote the colonization and growth of Bilophila wadsworthia, were elevated in ICP [73].

Zhan et al. also observed that the proportion of Escherichia/Shigella was enriched in ICP, as well as Parabacteroides [81]. Escherichia/Shigella was associated with lipid metabolism and liver enzymes in a study of nonalcoholic fatty liver disease [82]. Megamonas was reduced and Lactobacillus, Flavonifractor, Atopobium, and Turicibacter were enriched in ICP patients [81]. Lactobacillus was a bacterial group with functional BSH in mouse [83].

Moreover, Lactobacillus rhamnosus LRX01 was found to reduce susceptibility to lipopolysaccharide-induced inflammatory responses in offspring from CA-fed SD rats by inhibiting ileal FXR expression in a recent study [84]. Enrichment of

Escherichia/Shigella and other gram-negative bacteria was also detected in ICP rat offspring.

A recent metagenomic study [85] revealed that the gut microbiomes of ICP patients were primarily characterized by *Bacteroides fragilis* (*B. fragilis*), which inhibited FXR signaling via its BSH activity. According to the measurement of BA synthesis, such as by the levels of CYP7A1, CYP8B1 and CYP27A1, and bile excretion, such as by BSEP and MRP2, *B. fragilis* was responsible for the excessive BA synthesis and interruption of hepatic bile excretion, leading to the initiation of ICP. Overall, microbiome-modulated BA metabolism may have potential for ICP treatment.

In addition to the deconjugation of BAs through BSH activity mentioned above, there are other main BA biotransformation reactions catalyzed by gut microbiota, such as hydroxyl group oxidation, 7β-epimerization, 3β-epimerization, and 7-dehydroxylation. Finally, secondary BAs are produced, such as DCA, LCA, murideoxycholic acid (MDCA), UDCA, ω-muricholic acid (ω-MCA), and hyodeoxycholic acid [23], increasing BA diversity. Not only FXR signaling but also TGR5 signaling is strongly affected by gut microbiota, as secondary BAs such as LCA, DCA, and their conjugates are potent TGR5 activators.

In addition, tryptophan is essential among the metabolites at the interface between the host and gut microbiota. The predominant tryptophan metabolism pathways leading to kynurenine, indole and serotonin derivatives in the gut are controlled by these microbiota. In the kynurenine pathway, over 90% of tryptophan is a substrate. This pathway contains many metabolites, including kynurenine, 3-hydroxykynurenine, and 2-oxoadipic. In the indole pathway, indole and its intermediates are ligands of the aryl hydrocarbon receptor and play vital roles in intestinal homeostasis by regulating inflammatory signals [86]. Kynurenine, 3-hydroxykynurenine, 2-oxoadipic acid, and indole were significantly changed in ICP patients and associated with BA levels. Moreover, valeric acid, a gut microbial-specific metabolite, was reduced, while pantothenate (vitamin B5), a metabolite primarily produced by gut microbiota, was increased, in an ICP group [49]. This latter study implied that abnormal tryptophan metabolism may be associated with alterations in the intestinal flora in ICP.

In conclusion, the gut microbiota affect metabolomics in ICP not only through BAs but also through other metabolites, such as short-chain fatty acids [73] and tryptophan [49]. Further in-depth mechanistic studies are needed.

The gut microbiota with significant differential expression in the ICP microbiome studies are listed in Table 2.

Table 2 The gut microbiota with significant differential expression in ICP microbiome studies

Phylum	Firmicute (Li R, et al. 2020)	Bacteroidetes ↑; Firmicutes ↓ (Li GH,et al. 2020)	Bacteroidetes ↑; Firmicutes ↓ (Ovadia C,et al. 2020)	Proteobacteria (Zhan Q, et al. 2021)
Class	Bacilli, Gammaproteobacteria ↑ (Li R, et al. 2020)			
Order	Enterobacterials, Lactobacillales ↑ (Li R, et al. 2020)			
Family	Enterobacteriaceae, Leuconostocaceae, Streptococcaceae ↑ (Li R, et al. 2020)	Lactobacillaceae (Zhan Q, et al. 2021)		
Genus	Blautia; Citrobacter, Streptococcus ↑ (Li R, et al. 2020)	Parabacteroides, Bilophila, Bacteroides, Escherichia/Shigella ↑; Faecalibacterium, Blautia,Eubacterium hallii, Bifidobacterium ↓ (Li GH,et al. 2020)	Flavonifractor, Atopobium, Turicibacter, Parabacteroides, Lactobacillus, Escherichia/Shigella ↑; Megamonas ↓ (Zhan Q, et al. 2021)	Escherichia/Shigella ↑ (offspring from CA-fed SD rat) (Lin QX,et al. 2022)
Species	Streptococcus luteciae, [C.] methylpentosum ↑ (Li R, et al. 2020)			Bacteriodes fragilis, Klebsiella pneumoniae, Klebsiella variicola, Klebsiella quasipneumoniae, Weissella confusa, Citrobacter youngae, Enterobacter cloacae ↑; Coprococcus_catus ↓ (Tang B, et al. 2023)

Genetic factors

ABCB4 gene mutations have been found in ICP [87–93], whereas they were not associated with the response to UDCA therapy [94]. ABCB11 mutations had less of an effect on the pathogenesis of ICP [87] and raised the susceptibility to cholestasis of pregnancy [18].

Müllenbach et al. detected ATP8B1 mutations in ICP patients and suggested that susceptibility to ICP was related to the increase in biliary phospholipids [24]. A frameshift deletion in FGFR4 was also detected in ICP patients. It may lead to impaired enterohepatic feedback repression of hepatic BA synthesis via FXR and FGF19 [95]. TJP2 mutations and ANO8 mutations were detected in Han ICP patients by whole-exome sequencing [93].

The genes GABRA2, HLPT and KIFC3 play a role in the pathogenesis of pruritus, lipid metabolism and bile composition, and protein trafficking and cytoskeleton arrangement, respectively [96].

In addition, Du et al. [97] discovered roles in ICP for genes involved in the immune response, such as CXCL6, CXCL14 and IL-7R; genes associated with vascular endothelial growth factor (VEGF) signaling, such as FGF9, ITGB3 and VEGFC; and genes associated with GPCR signaling, including EGF, EGFR, and GNA14.

Complications of ICP and omics results

GCDCA, GCA, TC, glycodeoxycholic acid (GDCA) and TDCA were found to be markedly increased in ICP patients with MSAF and had a negative correlation with birth weight in a metabolomics study. TCDC was negatively and most strongly associated with birth weight [49]. Pearson correlation analysis showed that estrone sulfate levels were positively correlated with TBA levels and birth weights [49]. In another gut microbiota study, *B. fragilis* caused increased levels of TBA and was negatively correlated with birth weight, while supplementation of *B. fragilis*-treated mice with GDCA decreased their BA synthesis and increased their offspring's birth weight [85].

The elevated GCA, TBA, albumin and total bilirubin percentages taken together predicted preterm birth (PTB) in EICP, suggesting that high liver burden in the second trimester increases the risk of PTB. The TCA percentage predicted PTB in LICP(52).

Conclusion

BAs play a key role in the pathogenesis of ICP and are influenced by many aspects, such as enterohepatic circulation, placental BA homeostasis, and BA receptors. Various omics techniques have emerged as the techniques of bioinformatics have advanced, providing a new direction toward exploring the pathogenesis, diagnosis and treatment of ICP. For instance, high-throughput sequencing enables researchers to capture unknown targets from massive data. In studies of ICP, metabolomics provides insight into the role of bile acids, lipids, hormones, etc.; studies of the microbiome have dissected the role of gut microbes; and genomics studies have advanced our understanding of genetic factors in ICP. Most studies have focused on the analysis of biological information, and basic experimental verification is lacking. In addition, only a few different multiomics analyses have been applied. Combined multiomics analysis would be a systemic and in-depth approach to exploring this disease. For instance, genomic alterations may affect transporters of BAs, thereby causing changes in the BA profile in metabolomics, and ICP-induced changes in the relative abundance of BA-related compounds in metabonomics may influence the gut microbiota. Furthermore, emerging technologies such as single-cell omics and spatial methods have not been used in ICP. Overall, omics technology has yielded several significant findings and will keep playing a key role in the future.

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Data availability Not applicable.

Declarations

Conflict of interest Mi Tang have no relevant financial or non-financial interests to disclose. have no relevant financial or non-financial interests to disclose. Liling Xiong have no relevant financial or non-financial interests to disclose. Jianghui Cai have no relevant financial or non-financial interests to disclose. Jinzhu Fu have no relevant financial or non-financial interests to disclose. Hong Liu have no relevant financial or non-financial interests to disclose. Ying Ye have no relevant financial or non-financial interests to disclose. Li Yang have no relevant financial or non-financial interests to disclose. ShaSha Xing have no relevant financial or non-financial interests to disclose. Xiao Yang have no relevant financial or non-financial interests to disclose.

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