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INTRAHEPATIC CHOLESTASIS OF PREGNANCY

Genetic background, epidemiology and hepatobiliary consequences

Anne Ropponen

Academic Dissertation

To be presented by permission of the Medical Faculty of the University of Helsinki for public discussion in the Auditorium of the Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Haartmanninkatu 2, Helsinki, on May 19th, 2006, at 12 o'clock noon.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I *Savander M, *Ropponen A, *Avela K, Weerasekera N, Cormand B, Hirvioja ML, Riikonen S, Ylikorkala O, Lehesjoki AE, Williamson C, Aittomäki K. Genetic evidence of heterogeneity in intrahepatic cholestasis of pregnancy. Gut 2003;52:1025–1029.

II Ropponen A, Sund R, Ylikorkala O, Aittomäki K**.** A nation-wide study on intrahepatic cholestasis of pregnancy identifies new risk factors. (submitted)

III Ropponen A, Aittomäki K, Vihma V, Tikkanen MJ, Ylikorkala O. Effects of oral and transdermal estradiol administration on levels of sex hormone-binding globulin in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. J Clin Endocrinol Metab 2005;90:3431–3434.

IV Ropponen A, Aittomäki K, Tikkanen MJ, Ylikorkala O. Levels of serum C-reactive protein during oral and transdermal estradiol in postmenopausal women with and without a history of intrahepatic cholestasis of pregnancy. J Clin Endocrinol Metab 2005;90:142–146

V Ropponen A, Sund R, Riikonen S, Ylikorkala O, Aittomäki K. Intrahepatic cholestasis of pregnancy as an indicator of liver and biliary diseases: a population based study. Hepatology 2006;43:723–728

* Contributed equally to this work.

ABBREVIATIONS

ALAT	alanine aminotransferase
ASAT	aspartate aminotransferase
ATP	adenosine triphosphate
BRIC	benign recurrent intrahepatic cholestasis
BSEP	bile salt export pump
CA	cholic acid (3α7α12α-trihydroxy-5β-cholanoic acid)
CDCA	chenodeoksycholic acid (3α7α-dihydroxy-5β-cholanoic acid)
CRP	C-reactive protein
DCA	deoxycholic acid (3α12α-dihydroxy-5β-cholanoic acid)
E1	estrone
E2	estradiol
EPT	estrogen progestin therapy
ET	estrogen therapy
FIC1	a P-type ATPase, aminophospholipid transporter
FXR	farnesoid receptor X
GT	gammaglutamyl transferase
HELLP	hemolysis, elevated liver enzymes, low platelet count
HT	hormone therapy
ICD	International Classification of Diseases
ICP	intrahepatic cholestasis of pregnancy
IGF-1	insulin-like growth factor 1
IL-6	interleukin-6, cytokine
IVF	in-vitro-fertilization
LCA	lithocholic acid (3α-monohydroxy-5β-cholanoic acid)
LOD	logarithm of odds
MDR	multidrug resistance protein
MPA	medroxyprogesterone acetate
MRP	multidrug resistance associated protein
NETA	norethisterone acetate
NTCP	sodium-taurocholate cotransporter
OAT/OCT	transporters for the small organic compounds
OATP	sodium-independent organic anion transporting polypeptide
PBC	primary biliary cirrhosis
PCR	polymerase chain reaction
PFIC	progressive familial intrahepatic cholestasis
PSC	primary sclerosing cholangitis
SHBG	sex hormone-binding globulin
SHP	small heterodimeric partner

UDCA	ursodeoxycholic acid (3α7β-dihydroxy-5β-cholanoic acid)
ABCB4	gene for ATP-dependent phospholipid flippase, ABCB-family
ABCB11	gene for ATP-dependent BSEP-protein, ABCB-family
ABCC2	gene for multidrug resistance associated protein, ABCC-family
ABCG5/8	gene for halftransporters, ABCG-family
ATP8B1	gene for FIC1, a P-type ATPase
CYP7A1	gene for cholesterol-7α-hydroxylase (CYP7A1 enzyme)
CYP7B1	gene for oxysterol 7α-hydroxylase (CYP7B1 enzyme)
SLC	gene family for solute carrier proteins

ABSTRACT

Intrahepatic cholestasis of pregnancy (ICP) is the most common cholestatic liver disease during pregnancy. The reported incidence varies from 0.4 to 15% of full-term pregnancies. The etiology is heterogeneous but familial clustering is known to occur. Here we have studied the genetic background, epidemiology, and long-term hepatobiliary consequences of ICP.

In a register-based nation-wide study (n=1 080 310) the incidence of ICP was 0.94% during 1987–2004. A slightly higher incidence, 1.3%, was found in a hospital-based series (n=5304) among women attending the University Hospital of Helsinki in 1992–1993. Of these 16% (11/69) were familial and showed a higher (92%) recurrence rate than the sporadic (40%) cases. In the register-based epidemiological study, advanced maternal age and, to a lesser degree, parity were identified as new risk factors for ICP. The risk was 3-fold higher in women >39 years of age compared to women <30 years. Multiple pregnancy also associated with an elevated risk. In a genetic study we found no association of ICP with the genes regulating bile salt transport (*ABCB4, ABCB11* and *ATP8B1*).

The livers of postmenopausal women with a history of ICP tolerated well the short-term exposure to oral and transdermal estradiol, although the doses used were higher than those in routine clinical use. The response of serum levels of sex hormone-binding globulin (SHBG) to oral estradiol was slightly reduced in the ICP group. Transdermal estradiol had no effect on C-reactive protein (CRP) or SHBG. A number of liver and biliary diseases were found to be associated with ICP. Women with a history of ICP showed elevated risks for non-alcoholic liver cirrhosis (8.2 CI 1.9–36), cholelithiasis and cholecystitis (3.7 CI 3.2–4.2), hepatitis C (3.5 CI 1.6–7.6) and non-alcoholic pancreatitis (3.2 CI 1.7–5.7).

In conclusion, ICP complicates around 1% of all full-term pregnancies in Finland and its incidence has remained unchanged since 1987. It is familial in 16% of cases with a higher recurrence rate. Although the cause remains unknown, several risk factors, namely advanced maternal age, parity and multiple pregnancies, can be identified. Both oral and transdermal regimens of postmenopausal hormone therapy (HT) are safe for women with a history of ICP when liver function is considered. Some ICP patients are at risk of other liver and biliary diseases and, contrary to what has been thought, a follow-up is warranted.

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is the most common of familial cholestatic conditions. It usually manifests in the third trimester of pregnancy as skin itching and as elevation of the serum levels of bile acids and liver enzymes (Reyes 1997, Lammert et al 2000). The levels of liver transaminases are usually 2–10-fold higher than the normal range. Generally, ICP is considered to be harmless to the mother, but preterm birth (12–44%) (Fisk 1988, Rioseco et al 1994, Glantz et al 2004), fetal distress (10–44%) (Laatikainen et al 1984, Fisk 1988, Alsulyman et al 1996, Glantz et al 2004), and intrauterine fetal death (1–3%) (Laatikainen et al 1984, Fisk 1988, Alsulyman et al 1996) may ensue. Biochemical cholestasis resolves within a couple of days after delivery (Elferink 2003), but ICP may recur in 40–60% of subsequent pregnancies (Reyes 1997, Germain et al 2002). There has been a wide variation in the reported incidence of ICP in different countries (0.4–15%) (Abedin et al 1999, Locatelli et al 1999, Germain et al 2002); in Finland and Sweden the incidence is 0.54–1.5% (Laatikainen et al 1984, Berg et al 1986, Heinonen et al 1999).

ICP is thought to be the result of insufficient liver capacity to metabolize high amounts of placenta-derived sex steroids during pregnancy (Reyes 1997, Davidsson 1998). The familial occurrence of ICP in some cases suggests hereditary susceptibility (Holzbach et al 1983, Reyes et al 1993, Hirvioja et al 1993, Jacquemin 2001) and the increasing understanding of the genetic background of cholestatic diseases in general has aroused interest in the search for genes and mutations predisposing also to ICP. Moreover, the increased rate of cholelithiasis in these women may imply that ICP is not specific to pregnancy (Reyes 1997). This hypothesis is further supported by data showing that the high amounts of synthetic estrogens, eg. previously used oral contraceptives, can trigger symptoms and signs of cholestasis in women with a history of ICP (Kreek et al 1967, Adlercreutz et al 1964, Drill 1974, Reyes et al 1981).

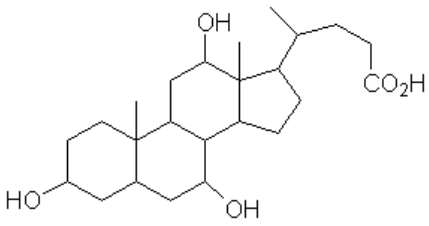
The present studies were designed to examine the genetic background, epidemiology and hepatobiliary consequences of ICP.

REVIEW OF THE LITERATURE

Intrahepatic cholestasis of pregnancy is not well defined in the medical literature and several names have been used for the condition. Icterus and pruritus gravidarum, recurrent jaundice of pregnancy, hepatosis gravidarum, and cholestasis of gravidarum have all been used as synonyms to the currently used intrahepatic cholestasis of pregnancy (ICP) (Reyes 1997) and obstetric cholestasis (Williamson et al 2004). Itching of healthy skin with elevated levels of liver transaminases and/or bile acids are the diagnostic criteria for ICP. In Finland, Sweden and Chile this disease entity has been well-known for a long time and in Finland at least three academic theses on ICP have been published previously (Ikonen 1964, Ylöstalo 1970, Heikkinen 1982).

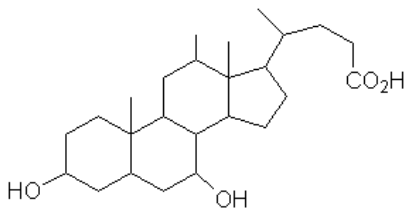
1. Physiology of bile acids

Bile acids facilitate excretion, absorption, and transport of fats and sterols in the intestine and liver (Trauner et al 2003). Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized from cholesterol by two pathways in the hepatocytes (Fuchs 2003). The two molecules are structurally very similar, differing by only one hydroxyl group at position 7 (Figure 1). Formation of CA through the classic pathway accounts for 90% of the total bile acid synthesis, which is up to 0.5g daily. The synthesis is regulated by the microsomal cholesterol-7α-hydroxylase (CYP7A1), which in turn is regulated by the availability of cholesterol and the concentrations of bile acids themselves. (Chiang 2003).



Cholic acid

3a7a12a-trihydroxy-5ß-cholanoic acid



Chenodeoxycholic acid

3a7a-dihydroxy-5ß-cholanoic acid

Figure 1. Structure of primarybile acids

An alternative pathway leads to the formation of CDCA, where the 7α-hydroxylation is preceded by the formation of oxysterols. These are metabolized by oxysterol 7α-hydroxylase (CYP7B1) enzyme (Chiang 2003).

The primary bile acids are conjugated in the hepatocytes with glycine or taurine to form bile salts, which are actively excreted to the bile through ATP-dependent membrane transporters (Fuchs 2003). The bile salts are then stored in the gallbladder as mixed micelles with phosphatidylcholine and cholesterol.

1.2. Enterohepatic circulation

The enterohepatic circulation ensures efficient use of synthesized bile acids with minimal daily loss (Figure 2). The pool of human bile salts, 2–4g, circulates 3–15 times per day resulting in 20–40g of daily excretion. Only 3–5% of bile salts (0.5g)

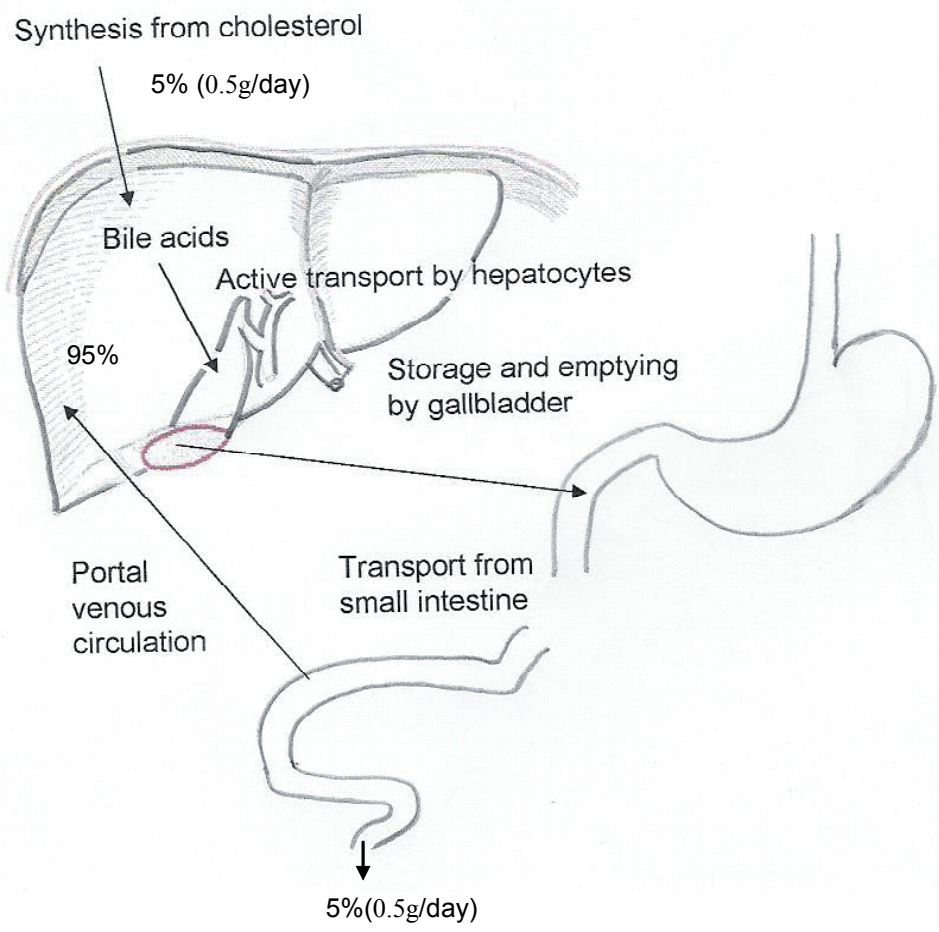


Figure 2. Enterohepatic circulation. Primary bile acids are synthesized in the liver and conjugated with glycine or taurine. Bile salts are excreted into bile and stored in the gallbladder. From the intestine bile acids are transported back to the liver via portal circulation.

are lost daily in faeces and are replaced by de novo synthesis from cholesterol. (Trauner et al 2003). The first step of the enterohepatic circulation is the excretion of bile from the gallbladder to the intestine. This secretion into the duodenum occurs after each meal due to vagal stimulation and release of cholecystokinin from the mucosa of the duodenum, which causes contraction of the gallbladder. (Chiang 2003). In the intestine CA and CDCA are converted to secondary bile acids, deoxycholic acid (DCA, 3α12α-dihydroxy-5β-cholanoic acid) and lithocholic acid (LCA, 3α-monohydroxy-5β-cholanoic acid), by bacteria (Chiang 2003) (Figure 3). Ursodeoxycholic acid (UDCA, 3α7β-dihydroxy-5β-cholanoic acid) is also formed by intestinal bacteria. It differs from CDCA by one hydroxyl group, which is in the β position at C-7. Only trace amounts of this bile acid are present in humans, whereas larger amounts are found at least in bears. (Trauner et al 2003, van Mil et al 2005).

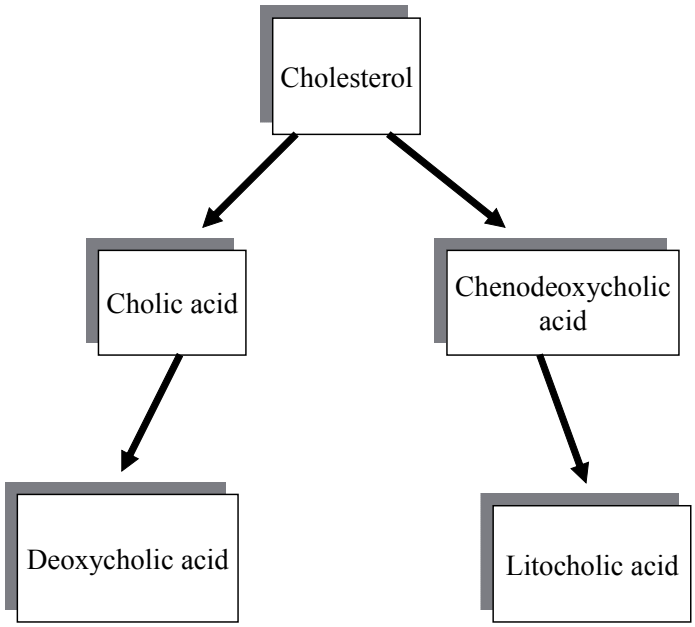


Figure 3. Primary and secondary bile acids are synthesized from cholesterol.

Conjugated bile acids are absorbed from the intestine by at least two carrier-mediated processes (van Mil et al 2005). Bile acids, returned to the liver in portal venous blood, are actively taken up by the liver and, once again, secreted into the bile thus completing the enterohepatic circulation. The large majority of bile salts (95%) are reabsorbed from the intestine (Pauli-Magnus et al 2005) (Figure 2 and Table 1). Equal amounts of conjugated CA and CDCA (80%), and conjugated DCA (10%) form the main bile acid pool and only traces of conjugated LCA and UDCA are present in the bile. Bile salts that escape from the enterohepatic circulation to the systemic circulation are filtered

Table 1. Bile acids in the enterohepatic circulation.

Amounts produced and circulated	
Total pool in body	2–4 g
Daily synthesis	0.5 g
Secretion	4–6 g/meal; 12–40 g/day
Recycling frequency	3–15 times/day
Concentrations	
Biliary ductuli	20–50 × 10^3μmol/l
Gallbladder	up to 300 × 10^3μmol/l
Venous blood	
Portal vein	20–50μmol/l
Systemic circulation	3–5 μmol/l

through the glomeruli in the kidneys and either reabsorbed from the tubules or excreted into urine. Normally the main excretion is through faeces, but in cholestatic conditions the amount excreted in urine increases (van Mil et al 2005). In blood bile salts are bound predominantly to albumin and high-density lipoproteins (60–85%). The ultimate goal of enterohepatic circulation is to ensure that bile salts are rapidly available in sufficient amounts when needed for digestion.

1.3. Transporter mechanisms

Hepatobiliary transport systems mediate hepatic uptake and biliary excretion of bile salts. Reduced or totally absent expression of these transporters has an important role in cholestasis (Chiang 2003) and for full comprehension of cholestatic disorders one must understand their function.

1.3.1. Transporters on basolateral membranes of hepatocytes

The basolateral membrane of the hepatocyte plays a key role in transporting bile acids and salts from venous blood to the liver (Figure 4). This is achieved by two major pathways. Firstly, the sodium-dependent sodium-taurocholate cotransporting polypeptide NTCP (a member of the solute carrier protein, the SLC-gene family, *SLC10A1*) accounts for more than 80% of conjugated, but for less than 50% of unconjugated bile salt uptake (Pauli-Magnus et al 2005). Secondly, the sodium-independent organic anion transporting polypeptides (OATPs) (members of the *SLC*-gene family) form another transporter system, which also transports bilirubin, bromsulphthalein, steroid sulphates and glu-

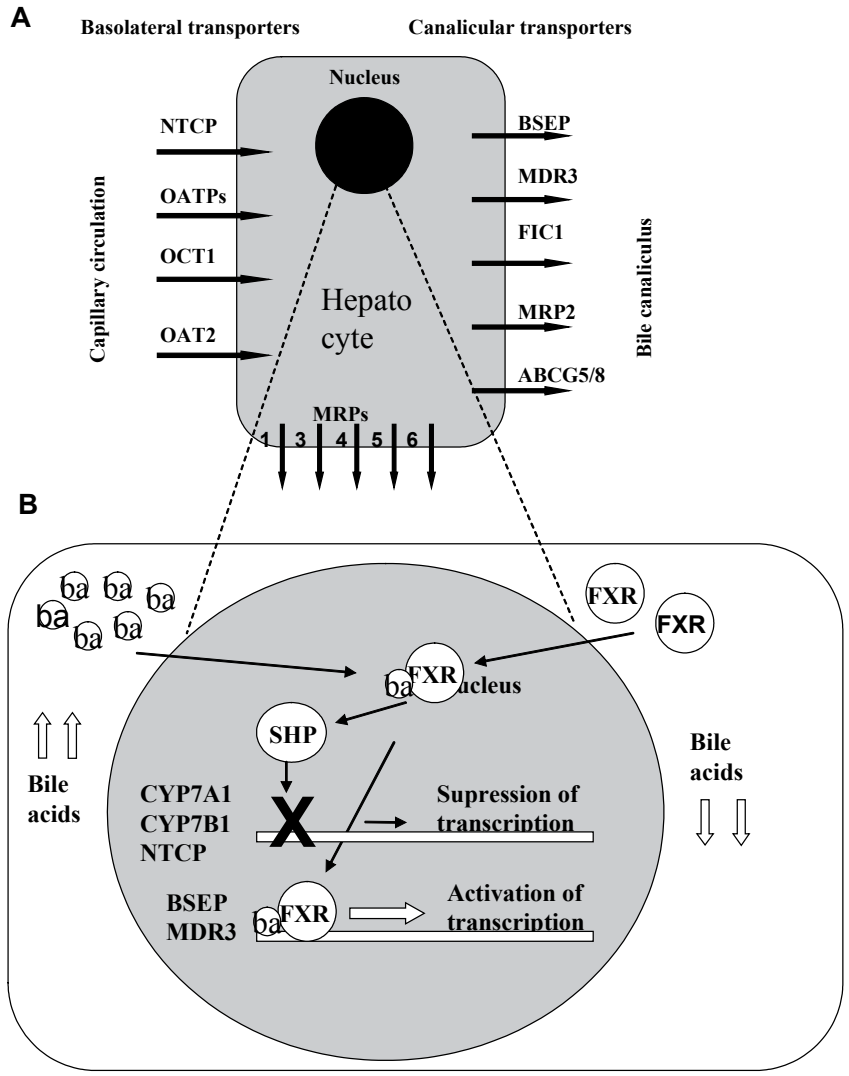


Figure 4.
A) Bile acid transporter proteins. Sodium-taurocholate transporting protein (NTCP), organic anion transporting polypeptides (OATPs) and transporter for small hydrophilic organic compounds (OAT/OCT) transport bile acids across the basolateral membrane into the hepatocyte. Multidrug resistance protein 3 (MDR3), bile salt export pump (BSEP), multidrug associated protein 2 (MRP2), a P-type ATPase FIC1, and halftransporters ABCG5/8 transport across canalicular membrane to the bile canaliculi.
MRPs are transporters of various organic anions, such as bile salts.

B) Schematic presentation of the regulation of intracellular bile acid concentration by nuclear receptors. When bile acid (ba) concentration rises in the hepatocyte, Farnesoid receptor X (FXR) binds to bile acids and inhibits via small heterodimeric partner (SHP) the transcription of CYP7A1 and CYP7B1 enzymes (inhibits synthesis of bile acids) and NTCP transporter (decreases uptake), but activates the transcription of BSEP and MDR3 (increases excretion). As the result, bile acid concentration decreases in the hepatocyte.

curonides, neutral steroids, and conjugated and unconjugated bile salts (Pauli-Magnus et al 2005). Bile acids and salts are highly toxic to cells (van Mill et al 2005) and they are bound to intracellular proteins in the hepatocytes (Ferenci et al 2002), although it is not known exactly how they are transported there. Anyway, the basolateral transporters regulate the uptake of bile salts from venous blood to hepatocytes.

1.3.2. Transporters on canalicular membranes of hepatocytes

The canalicular membrane of the hepatocyte faces the bile ductulus (Figure 4). The transporter proteins on the canalicular membrane belong to the family of ATP-dependent P-glycoproteins (ATP-Binding-Cassette, ABC-superfamily) (Lee et al 2000). These include several subgroups of transporter proteins, such as multidrug-resistance proteins (*ABCB*-gene family), multidrug-resistance associated proteins (*ABCC*-gene family), and ABC-halftransporters (*ABCG*-gene family) (Pauli-Magnus et al 2005).

The bile salt export pump (BSEP, *ABCB11*) is primarily responsible for transporting the conjugated bile salts from the hepatocyte to the bile canaliculi. The multidrug resistance protein 3 (MDR3, *ABCB4*) functions as a phospholipid flippase translocating phospholipids from the inner to the outer leaf of the canalicular membrane (Pauli-Magnus et al 2005) (Figure 4). A P-type ATPase, the FIC1-protein (*ATP8B1*), is involved in bile acid transport. Its function is not well understood, but it is an ATP-dependent aminophospholipid transporter and thought to play a role in the regulation of the bile acid pool. (Pauli-Magnus et al 2005). The multidrug resistance-associated protein 2 (MRP2, *ABCC2*) is the only member of this MRP subgroup which is located on the canalicular membrane while the other MRPs are on the basolateral membrane (Pauli-Magnus et al 2005). It transports bile salt conjugates, bilirubin diglucuronide, and glutathione conjugates. Halftransporters ABCG5 and ABCG8 are also expressed in the canalicular membrane and participate in cholesterol and plant sterol excretion (Yu et al 2002). Taken as a whole, the basolateral and canalicular membranes contain many transporter proteins which regulate bile acid and cholesterol homeostasis in man and which, if defective or absent, lead to cholestasis.

1.3.3. Intestinal transporters

Bile acids and salts are actively transported in the intestine. In the terminal ileum, the apical membrane of the enterocytes contains sodium-dependent bile salt transporters (*SLC10A2*). In the enterocytes, bile acid-binding protein transports bile acids to the basolateral membrane (van Mil et al 2005).The mechanism of bile salt transport to the venous blood and portal circulation is unknown (Pauli-Magnus et al 2005). A multidrug resistance-associated pro-

tein, MRP3, is expressed in the basolateral membrane of enterocytes (Pauli-Magnus et al 2005), and is a candidate transporter for this.

1.3.4. Regulation

The intracellular concentration of bile acids in the hepatocytes is tightly regulated as bile acids are highly toxic due to their detergent properties. The synthesis and excretion of bile acids and salts are controlled by their concentration in blood and hepatocytes. In response to high bile acid concentration, both the synthesis and absorption in the hepatocyte decrease while the secretion into the bile increases (van Mill et al 2005). Regulation of these processes is mediated by transcription factors called nuclear hormone receptors, which can activate or inactivate the transcription of relevant genes (Figure 4). Farnesoid X receptor (FXR), when bound to bile acids, activates the transcription of short heterodimeric partner (SHP), which leads to inactivation of the rate limiting step in bile acid synthesis (CYP7A1 and CYP7B1 enzymes) (Figure 4). At the same time, bile salt absorption decreases from the capillary venous blood, because SHP also inhibits the expression of the NTCP-pump in the basolateral membrane. (Pauli-Magnus et al 2005, van Mil et al 2005). In contrast, the transcription of BSEP and MDR3 is increased by FXR, enhancing the excretion of bile acids. Thus, the concentration of bile acids in the hepatocyte is decreased by all these mechanisms. On the other hand, liver X Receptor reacts to high cholesterol levels in the hepatocyte and CYP7A1 enzyme is induced to convert cholesterol to bile acids. (van Mil et al 2005). Summing up, the homeostasis of bile acids and cholesterol is efficiently regulated in the hepatocytes, but the complex regulatory mechanisms are still incompletely understood.

2. Intrahepatic cholestasis

Cholestasis is defined as an impairment of bile secretion due to intrahepatic or extrahepatic causes. Any diseases which lead to failure in the synthesis or decreased excretion of bile acids within the liver can result in cholestasis (Balistreri et al 2005). Extrahepatic cholestasis is caused by any obstructing factor in the bile ducts outside the liver, of which cholelithiasis and pancreatic tumors are the two most common. Normally the ratio of bile salts bound to glycine or taurine is 3:1, but in cholestasis the concentrations of sulphate and glucuronide conjugates increase (Lammert et al 2000). Likewise, excretion of bile salts through the kidneys increases in cholestasis, which may explain the increased amounts of sulphate and glucuronide conjugates in urine (Trauner et al 2003). The ratio of CA to CDCA is normally less than 1.5, but it increases in cholestatic conditions (Lammert et al 2000). The increased concentration of bile acids in cholestasis inhibits their synthesis. This in turn leads to the accumulation of

cholesterol and hypercholesterolemia may ensue. (Chiang 2003). Lipoprotein X is an abnormal, low density lipoprotein which is present only in patients with cholestasis (Jacquemin 2001). These changes are typical to cholestasis, regardless of the causative factor (Chiang 2003), and explain how the synthesis and homeostasis of bile acids and cholesterol are intertwined with each other and contribute to the clinical phenotypes.

2.1. Intrahepatic cholestatic diseases

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are the major chronic cholestatic diseases. Their prevalence is approximately 5 and 3 per 100 000, respectively, and over 90% of PBC patients are women (Kowdley 2000). Both diseases progress slowly to cirrhosis. It is established that inflammatory factors are important in PBC and PSC and that in PSC the large and medium sized bile ductuli are destroyed while in PBC the small bile canaliculi are affected (Kowdley 2000). The risk of PBC is nearly 100-fold elevated in the first-degree relatives of patients (Parikh-Patel et al 1999). No genetic defect has been found so far either in PBC or PSC (Pauli-Magnus et al 2005).

Sepsis and several viruses, such as Ebstein-Bar, cytomegalo, herpes and hepatitis, may also cause cholestasis (Elferink 2003, Hinedi et al 2003). This may be a reflection of infection-inflammation cascade. In hepatitis C the down-regulation of the MRP2 expression may also be involved. (Hinoshita et al 2001).

The mechanism, by which drugs and alcohol induce cholestasis, is not known. They may inhibit the BSEP-pump, thus impairing the excretion of bile salts from the hepatocyte (Pauli-Magnus et al 2005), but they also impair the expression of NTCP and OATPs, decreasing the uptake of bile salts from the systemic circulation (Zollner et al 2001). As briefly described above, much is known of the causes and development of intrahepatic cholestasis, but the regulatory mechanisms behind these phenomena are still poorly understood.

2.2. Familial cholestatic diseases

Familial intrahepatic cholestases comprise a group of diseases which are mostly quite rare (Table 2). These include defects of synthesis and transport of bile acids (Jacquemin 2001, van Mil et al 2005) and some other rare conditions (Table 2). Mutations behind this heterogeneous group of familial diseases have been identified in recent years (Pauli-Magnus et al 2005). Some of them (such as benign recurrent intrahepatic cholestases, BRICs) are mild with long, symptom-free periods intervening with recurrences. Others are serious illnesses (such as progressive familial intrahepatic cholestases, PFICs) leading to progressive liver failure and cirrhosis and may necessitate liver transplantation already in childhood (Jacquemin 2001). Defects in the canalicular transporters (Figure 4) include three subtypes of recessively inherited progressive familial intrahepatic

Table 2. Syndromes of familial intrahepatic cholestasis.

Disorders of bile acid synthesis; Phenotype	Gene locus	Gene	Defect
Hyperlipidemia, premature coronary and peripheral vascular disease, and premature gallstone disease	8q21.13	*CYP7A1*	CYP7A1 deficiency
Neonatal cholestasis and cirrhosis	8q21.3	*CYP7B1*	CYP7B1 deficiency
Intrahepatic cholestasis, jaundice, pale stools, dark urine	7q32–33	*AKR1D1*	3β-Δ5-C27-hydroxysteroid oxidoreductase deficiency
Xanthomas and cardiovascular problems	2q33qter	*CYP27A1*	Cerebrotendinous xanthomatosis
Adult onset sensory motor neuropathy, accumulation of pristanic acids and bile acid intermediates	15p13.2–5q11.1	*AMACR*	2-Methylacyl CoA racemase deficiency
Hepatomegaly, developmental defects, hypotonia, seizures	5q2	*HSD17B4*	D-bifunctional protein deficiency
Defects of bile acid synthesis/tight junction			
Familial hypercholanemia (FHCA)	9q12–13	*TJP*	Defective tight junction protein
Fat malabsorption, vitamin K-deficiency, sometimes cholestasis and chronic hepatitis	9q22–23	*BAAT*	Defective tight junction protein

Disorders of bile acid synthesis; Phenotype	Gene locus	Gene	Defect
Defects in the canalicular transport			
Progressive familial intrahepatic cholestasis type 1 (PFIC1)	18q21-22	*ATP8B1*	FIC1 protein
Benign recurrent intrahepatic cholestasis type 1 (BRIC1)	18q21–22	*ATP8B1*	FIC1 protein
Progressive familial intrahepatic cholestasis type 2 (PFIC2)	2q24	*ABCB11*	Bile salt export pump
Benign recurrent intrahepatic cholestasis type 2 (BRIC2)	2q24	*ABCB11*	Bile salt export pump
Progressive familial intrahepatic cholestasis type 3 (PFIC3)	7q21	*ABCB4*	Multidrug resistance protein 3
Intrahepatic cholestasis of pregnancy (ICP)	7q21 18q21–22	*ABCB4* *ATP8B1*	Multidrug resistance protein 3 FIC1 protein
Other syndromes of familial intrahepatic cholestasis			
Atrhrogryposis, renal tubular dysfunction, and cholestasis (ARC-syndrome)	15q26.1	*VSP33B*	Abnormal intracellular protein trafficking
Lymphoedema-cholestasis syndrome (LCS)/ Aagenaes syndrome (Neonatal intrahepatic cholestasis and lymphoedema)	15q	*7*	Not known
North American Indian childhood cirrhosis (NAICC) (Neonatal jaundice, biliary cirrhosis	16q22	*Cirhin*	Cirhin deficiency

cholestases (PFIC1, PFIC2 and PFIC3) and two subtypes of benign recurrent intrahepatic cholestasis (BRIC1 and BRIC2). Cholestasis presenting already during the first year of life is characteristic for all PFIC types (Table2) (Jacquemin 2001). In PFIC types 1 and 2 concentrations of bile salts in serum are high, while the levels of cholesterol and gamma-glutamyl transferase (GT) are normal. Steatorrea, diarrhea and jaundice belong to clinical features of PFIC1 (Pauli-Magnus et al 2005). These symptoms are not present in patients with PFIC2. Mutations in the *ATP8B1* gene encoding the FIC1 transporter cause PFIC type 1 and cause total or almost total absence of functional FIC1 protein on the canalicular membrane (Figure 4) (Pauli-Magnus et al 2005, van Mil et al 2005). The same protein is also expressed on the cell membranes in the intestine, pancreas and biliary tract cholangiocytes. This may explain the occurrence of pancreatitis and diarrhea in these patients (Pauli-Magnus et al 2005). Mutations in the BSEP, coded by *ABCB11,* cause decreased bile salt export to the bile canaliculus in PFIC type 2 patients.

Only the liver is affected in PFIC type 3 and these patients have high levels of gamma GT (Jacquemin 2001). Phospholipids, which are important in the formation of micelles in biliary canaliculi, are partly or totally missing from bile in this disease depending on the type of mutation in the *ABCB4* gene encoding the MDR3 protein. Bile ducts are thus proliferated and typically these patients have jaundice, itching, hepatosplenomegaly, portal hypertension and ultimately liver function fails (van Mil et al 2005).

It has been thought that BRIC diseases are clinically benign diseases. They typically begin in adulthood with recurrent periods of cholestasis. Mutations in BRIC types 1 and 2 are in the same genes, *ATP8B1* and *ABCB11*, which cause PFIC types 1 and 2. The differences of the mutations with varying residual function seem to explain the varying phenotypes especially in PFIC1 and BRIC1 patients. (Pauli-Magnus et al 2005). A later developing progressive cholestasis has also been reported in patients who were thought to have the benign type of the disease. Cholelithiasis is associated with *ABCB11* gene mutations in BRIC2 patients. As in BRIC1 some patients with BRIC2 manifest a more aggressive type of disease later in life (van Mil et al 2005). Not all families segregating the BRIC phenotype have mutations in these two genes, suggesting a third locus (Pauli-Magnus 2005). As we have seen different mutations in genes encoding the transporting proteins of bile acids lead to cholestasis.

3. Liver during pregnancy

Although human pregnancy is accompanied by significant changes in cardiovascular and other physiology, the liver and its function show relatively small alterations in normal pregnancy (Scherlock 1985). The size of the liver and its blood flow do not differ from those in the non-pregnant state. In liver biopsy, only mild lymphocytic infiltration of the portal zones can be seen, but there are no changes specific for pregnancy. (Scherlock 1985). However, teleangiectasias and palmar erythema, which can be markers of liver dysfunction in non-pregnant subjects, may appear in up to 60% of normal pregnancies without any evidence of liver disease (Knox 1998). The increasing levels of progesterone may relax the smooth muscle of the gallbladder, which does not contract efficiently during pregnancy (Riley 1992).

Changes seen in the function of the liver during normal pregnancy include reduced clearance of bromsulphtalein during the last trimester (Simcock et al 1967, Fulton et al 1983). Additionally the synthesis of albumin decreases progressively, and its level in serum is around 30% lower at term as in non-pregnant women; this can of course be partly due to the relative hemodilution (Branch 1992). Likewise, the synthesis of globulins decline but not as much as albumin and therefore the albumin/globulin ratio decreases. Estrogen increases the synthesis of several proteins in the liver such as fibrinogen and ceruloplasmin, and the binding proteins for corticosteroids, sex steroids, and thyroid hormones. Increased fibrinogen concentrations result in the elevation of the erythrocyte sedimentation rate. (Branch 1992). Total and LDL-cholesterol levels increase by approximately 25% and 34%, respectively, from baseline to week 36 during normal pregnancy (Amundsen et al 2006). The levels of bile acids and liver transaminases do not change during normal pregnancy, but serum alkaline phosphatase levels rise mainly due to leakage of placental alkaline phosphatase (Laatikainen 1977, Carter 1991, Bacq et al 1996). However, the bile acid pool in fetal serum at term differs from the maternal pool. During normal pregnancy the main bile acid in the fetus is CDCA (Laatikainen 1977). Because in maternal blood the ratio of CA to CDCA is less than 1.5, the placenta maintains a concentration gradient for these bile acids between maternal and fetal plasma. Recently, some bile acid transporter proteins, namely MDR3, FIC1, and two members of OATP-protein family, have been found to be expressed in placenta (Patel et al 2003). Although OATPs and FIC1 are down-regulated in the third trimester, MDR3 protein expression is up-regulated four-fold compared to the first trimester of normal pregnancy (Patel et al 2003).

3.1. Intrahepatic cholestasis of pregnancy

The most common form of intrahepatic cholestatic condition in general is ICP and it is the most common liver disorder during pregnancy.

3.1.1. Epidemiology

The incidence of ICP shows large variation between different countries and populations (Lammert et al 2000). In Finland and Sweden, ICP occurs in approximately 1.0–1.5% of pregnancies (Laatikainen et al 1984, Berg et al 1986) while in other European countries the incidences have been lower e.g. in France and Italy 0.4–1% (Reyes 1997, Locatelli et al 1999, Roncaglia et al 2002). In the United Kingdom, ICP affects only 0.6% of pregnancies in white Caucasians, but 1.4% of pregnancies of Indian and Pakistani origin (Abedin et al 1999), suggesting ethnic origin as one determinant of the risk. The highest incidence of ICP (14%) has been reported from Chile (Reyes 1997), but a much lower incidence (2–4%) was found in the latest report (Germain et al 2002). The varying incidences may be explained by differences in diagnostic criteria, genetic background, or in the environment.

Multiple pregnancy increases the risk of ICP 5-fold (Gonzalez et al 1989, Glantz et al 2004). The recurrence of ICP has been noted in 40–60% of patients (Reyes 1997). The data available so far imply that there are three risk factors for ICP multiple pregnancy, positive family history and a previous pregnancy with ICP (Davison 1998).

3.1.2. Pathogenesis

In spite of extensive studies, the cause of ICP is still unknown. Yet it is commonly accepted that ICP could be the result of a relative incapacity of the liver to metabolize the high amounts of placenta-derived steroids during pregnancy (Reyes 1997). This is also supported by data showing that multiple pregnancies with higher steroid loads predispose to ICP (Laatikainen et al 1984, Gonzalez et al 1989, Glantz et al 2004). Accordingly, the previously used high-dose oral contraceptives have been shown to trigger cholestasis in women with a history of ICP (Kreek et al 1967, Adlercreutz et al 1964, Drill 1974, Reyes et al 1981). The liver histology in ICP reveals only dilated bile canaliculi without little or no evidence of hepatocellular change and no inflammatory reaction.

3.1.3. Genetics

A genetic background is suggested by the familial occurrence of ICP. Several published pedigrees support the dominant mode of inheritance (Holzbach et al 1983, Hirvioja et al 1993, Eloranta et al 2001). The observation that heterozygous mothers of children with recessively inherited progressive familial intrahepatic cholestasis type 3 (PFIC3) had experienced ICP during pregnancy implies that the genetic background, namely *ABCB4* gene mutations, exists for ICP in these families (Dixon et al 2000, Jacquemin 2001, Gendrot et al 2003, Lucena et al 2003, Müllenbach et al 2003). A tendency to gallstone disease in these heterozygous parents could be explained by high cholesterol and low

phospholipid concentrations in bile. A tendency to develop ICP was proposed to be associated with a specific *ABCB11* allele (Eloranta et al 2003), although later studies did not confirm this (Painter et al 2004). Recently, several variants of the *ATP8B1* gene (encoding the FIC1 protein) have been detected in women with ICP (Painter et al 2005, Müllenbach et al 2005), but the significance of these findings is still uncertain. Presently ICP is classified as a cholestatic condition with defective bile acid transport, as is the case for those few patients in whom the molecular genetic background has been identified, but other disease mechanisms are also possible.

3.1.4. Clinical picture and treatment

Typically ICP manifests in the third trimester with itching, which starts on the soles and palms. Subsequently it extends to the extremities and trunk, and is usually aggravated during the night disturbing sleep. Levels of liver transaminases in serum are usually 2–10-fold increased and bile acids may be up to 100 times higher than the normal range (Reyes 1992). Typically, the levels of CA are at least twice as high as the levels of CDCA (Heikkinen et al 1981, Brites et al 1998) and taurine conjugates are increased. The glycine/taurine ratio in maternal blood during pregnancy with ICP is half of that in normal pregnancy (Brites et al 1998). Total and LDL-cholesterol concentrations are higher during pregnancy with ICP than in normal pregnancy (Dann et al 2006).

No anatomical changes have been detected in the liver or biliary tract compared to non-pregnant findings in ultrasonography. Biochemical cholestasis resolves in a couple of days after delivery (Elferink 2003).

To the mother ICP does not impose a risk (Reyes 1997). However, it is associated with increased fetal risks such as preterm birth (12–44%) (Fisk et al 1988, Rioseco et al 1994, Glantz et al 2004), fetal distress (10–44%) (Laatikainen et al 1975, 1984, Fisk et al 1988, Alsulyman et al 1996, Glantz et al 2004), or death of the fetus (1–3%) (Laatikainen et al 1975, Fisk et al 1988, Alsulyman et al 1996). It is not known by which mechanism ICP endangers fetal wellbeing, but in autopsies of stillbirth, signs of acute asphyxia (petechial bleeding in lungs, pericardium, and adrenal glands) are seen. Placental histology reveals only non-specific changes. (Williamson et al 2004). It has been proposed that maternal levels of bile acids exceeding 40μmol/l could be used as a marker of possible fetal risk (Glantz et al 2004). Although there is discrepancy over whether the fetal distress is the result of high levels of bile acids in the fetal blood (Laatikainen 1977, Heikkinen et al 1980, Shaw et al 1982, Alsulyman et al 1996, Laatikainen et al 1984), the levels of bile acids in fetal serum and amniotic fluid are higher in ICP than in normal pregnancies (Laatikainen et al 1978, Rodrigues et al 1999). One mechanism by which bile acids conjugated with taurine could endanger fetal well-being is their arrhythmogenic effect on the fetal cardiac myocyte function (Gorelik et al 2004).

Various medications, such as antihistamines and benzodiazepines, have been used to relieve the itching. These do not affect the bile acid levels in the serum. Phenobarbital, when used for treatment, can induce the hepatic microsomal enzymes and thereby it may increase the excretion of bile acids (Davidsson 1998). Intravenous S-adenosylmethionine (Frezza et al 1990, Ribalta et al 1991, Nicastri et al 1998), oral dexamethasone (Hirvioja et al 1992, Glantz et al 2005), cholestyramine (Laatikainen 1978, Heikkinen et al 1982), and guar gum (Riikonen et al 2000) have been shown to reduce the levels of bile acids or to relieve itching. Most studies have been small and there is insufficient evidence of the benefits (Burrows et al 2001).

A naturally occurring hydrophilic bile acid UDCA modifies the bile acid pool. It is widely used in treatment of cholestatic diseases in general (James 1990). The concentrations of CA and CDCA decrease during UDCA treatment both in maternal and cord blood, in amniotic fluid and in colostrum (Brites et al 1998, Berkane et al 2000, Mazzella et al 2001) but not in the meconium. In enterohepatic circulation UDCA replaces the CA and CDCA and CA/CDCA ratio returns to the same level as in normal pregnancy (Brites et al 1998). During treatment, the concentration of UDCA increases in the maternal serum, but only very slightly in the amniotic fluid (Mazzella et al 2001). In hepatocytes UDCA binds to FXR thereby decreasing the synthesis of bile acids and increasing excretion.

Tauroconjugates of bile acids, which are increased in ICP patients (Brites et al 1998), are also decreased during UDCA treatment. No maternal or fetal side effects have been described (Davies et al 1995, Davidsson 1998, Burrows et al 2001).

Fetal surveillance is necessary in pregnancies with ICP, at least at an outpatient clinic, as is the use of UDCA to lower the bile acid levels. Clinically, the levels of liver transaminases and bile acids are determined routinely once or twice weekly and fetal cardiotocography recorded weekly or even daily, depending on the bile acid levels in the maternal blood. Yet a normal fetal heart rate does not guarantee fetal well-being (Alsulyman et al 1996, Sentilhes et al 2006). As clinicians do not have a totally reliable test for fetal risks, labour is often induced 1-2 weeks before term.

3.1.5. Associated hepatobiliary disorders

Although it has been established that a history of ICP predisposes to gallstone disease (Reyes 1997), other hepatobiliary consequences of ICP are scarcely known. It is of course possible that in some ICP cases an early form of PBC may have been present and misdiagnosed as ICP (Leevy et al 1997, Reyes 1997, Lucena et al 2003). It has also been reported that the incidence of ICP is elevated in women with hepatitis C (Locatelli et al 1999) but this finding has not been confirmed by others. Thus, except for an elevated risk of cholelithiasis, al-

most no data exist on the subsequent hepatobiliary or other diseases in women with a history of ICP.

3.2. Other liver diseases and pregnancy

Many chronic liver diseases do not prevent pregnancies and therefore, pregnancies may be accompanied by these. Pruritus, nausea, jaundice, and acute abdominal pain during pregnancy all necessitate the testing of liver function and a search for the cause.

Pregnancy does not alter the course of hepatitis A, B or C (Floreani et al 1996, Hunt et al 1999). Hepatitis C, however, may be associated with ICP or an ICP-like condition (Locatelli et al 1999). Liver cirrhosis impairs fertility and, in case of a pregnancy, the risk of spontaneous abortion, hypertension, pre-eclampsia, and preterm birth may be increased (Armenti et al 2000). Pregnancy may also deteriorate the prognosis of cirrhosis (Marinaccio et al 1992, Armenti et al 2000). Inherited disorders of bilirubin metabolism are characterized by elevated levels of serum conjugated or unconjugated bilirubin and they may further elevate during pregnancy especially in Dubin-Johnson syndrome, which is caused by defective function of the MRP2 (Knox 1998, Keppler et al 2000). Pregnancy does not, however, impair the liver function or prognosis of these patients.

Cholelithiasis may naturally occur at any time during pregnancy, and pregnancy itself predisposes to cholelithiasis. It is known that 6–12% of women have asymptomatic gallstones immediately after delivery (Valdivieso et al 1993, Hunt et al 1999). Severe pre-eclampsia may also be accompanied by changes in the liver function (Knox et al 1996). Hemolysis, elevated liver enzymes, and a low platelet count constitute the HELLP syndrome accompanied with other symptoms of pre-eclampsia.

Acute fatty liver of pregnancy affects 1 in 13 000 pregnancies and occurs typically during the third trimester (Knox et al 1996, Hunt et al 1999). A hematoma or rupture of the liver are encountered very rarely (1 in 40 000–250 000 pregnancies) (Hunt et al 1999). Acute hemorrhage and subcapsular hematoma of the liver are usually associated with severe pre-eclampsia (80%), but tumors and abscesses can also predispose to their occurrence (Knox 1998).

Severe liver diseases during pregnancy are not common, but they are clinically important as many of them are serious diseases which may even have fatal outcomes.

4. Postmenopausal hormone therapy and liver proteins

With increasing age the size of the liver and also its blood flow decreases and the repair of damaged cells is slower than in younger people. The synthesis of albumin does not change and the levels of liver transaminases stay within the normal range. The production and flow of bile decrease and this may contribute to the increased risk of gallstone formation. (Vuoristo 2001).

Hormone therapy (HT), estrogen alone (ET) or in combination with progestin (EPT), is commonly used to alleviate postmenopausal vasomotor symptoms (MacLennan et al 2002). These regimens are usually administered orally or transdermally, although estrogens can be given also intranasally, subcutaneously or intramuscularly (Samsioe 2002). Estrogens and progestins given orally undergo the first pass metabolism in the liver, whereas when given parenterally these hormones escape this step (Nachtigall 1995). After oral administration of estradiol (E2) approximately 70% is metabolized in the liver to estrone (E1), which is the main circulating estrogen during oral treatment. Further breakdown happens through two major pathways, which result in metabolites with decreasing activity, namely estriol (E3) and cathecol-estrogens (Samsioe 2002). In the liver E1 and E2 are conjugated mainly to glucuronide and sulfate and excreted through bile to the intestine, where they are deconjugated and 80% are reabsorbed into blood. Only a minor proportion (1–2%) of E1 and E2 circulate as free hormones, because they are bound to SHBG (40%), and to albumin (58%) (Dunn 1981, Plowchalk 2002).

The main circulating estrogen during transdermal E2 administration is E2 (Samsioe 2002). Transdermal HT does not affect the hepatic metabolism (Serin et al 2001). Whether postmenopausal HT affects liver function and the production of CRP and SHBG in women with a history of ICP is unknown.

4.1. Sex hormone-binding globulin

The synthesis of SHBG occurs in the liver (Jänne et al 1998). Plasma SHBG regulates the bioavailable fraction of steroids and also their access to target cells (Hryb et al 1990, Rosner et al 1991, Kahn et al 2002). The gene coding for SHBG resides in chromosome 17 and hepatocyte nuclear factor-4 controls the transcription (Jänne et al 1998). Mutations in this gene result in decreased excretion of SHBG (Hogeveen et al 2001, 2002). Estrogen and thyroid hormones increase, while androgens decrease the synthesis of SHBG (Anderson 1974, Loukovaara et al 1995, Nachtigall et al 2000). Hyperinsulinemia, insulin-like growth factor-1 (IGF-1) and hyperandrogenism are associated with low circulating SHBG (Pugeat et al 1995, Kalme et al 2003, Hogeveen et al 2002). Falling levels of estrogens are thought to explain the decrease in the levels of SHBG after menopause (Sarrel 2002).

Use of oral ET elevates the concentrations of SHBG (Samsioe 2002). Transdermal ET, however, has no effect on SHBG levels according to most studies (Steingold KA et al 1991, Nachtigall et al 2000, Samsioe 2002), although small increases in SHBG levels have been reported (Kramer et al 2003). The effect of a progestin on serum SHBG levels depends on the androgenicity of a given therapy (Nugent et al 2003). Androgenic progestins, e.g. norethisterone acetate, decrease the synthesis of SHBG, whereas cyproterone acetate and dydrogesterone have no effect (Nugent et al 2003). Changes in SHBG concentrations during HT use may be of clinical significance because the higher the level of SHBG the smaller the biologically active free fraction of estrogen (Samsioe 2002). This may determine, at least in part, the clinical consequences of estrogen use, both desired and undesired.

4.2. C-reactive protein

During the last few years CRP has become a focus of intense research in menopause. This protein has been used for years as a marker of infection or inflammation. In the tissue, infection causes the release of cytokines which then enter the liver stimulating the synthesis of CRP (Straub et al 2000). Recent data have, however, suggested that even subclinical elevations in the concentration of CRP may predict the risk of cardiovascular disease (Koenig et al 1999, Ridker et al 2000). This effect could be mediated through activation of the complement cascade in atherosclerotic plaques in blood vessels, which may ultimately result in a thrombosis-like event in arterial walls (Lagrand et al 1999).

Long-term use of oral HT has been associated with elevated levels of CRP whereas transdermal HT has had no effect (Table 3). It has been thought that this CRP rise may be responsible for the negative effects of HT in the trials where oral HT has been given either for primary (Manson et al 2003) or secondary (Hulley et al 1998) prevention of cardiovascular diseases. It is difficult to explain why oral HT raises CRP because the synthesis of cytokine interleukin 6 (IL-6), the main stimulator of CRP synthesis, does not increase during oral HT. (Lakoski et al 2005). Although much data have been collected on CRP during HT use, we still do not know how soon the effect of oral HT becomes detectable or whether it is strictly dependent on the dose of estrogen (Lakoski et al 2005). Moreover, the effect of progestin on CRP is less clear and, finally, no data exist on the impact of HT on CRP in women with a history of ICP.

Table 3. Studies evaluating the effect of oral and transdermal hormone therapy on C-reactive protein with different progestins.

Study	Estrogen + progestin	Dose/day	**	months	CRP
van Baal	Estradiol	2mg	O	1–3	↑
1999	Estradiol + Dydrogesterone (14days/monthly) or Trimegestone (continuous)	2mg 10mg 0.5mg	O O O	1–3	↑
Cushman	Conjugated estrogens	0.625mg	O	12–36	↑
1999	Conjugated estrogens + MPA (12days/monthly) or MPA (continuous) or micronized progesterone (12days/monthly)	0.625mg 10mg 2.5mg 200mg	O O O O	12–36	↑
Wakatsuki	Conjugated estrogens	0.625mg	O	3	↑
2002	Conjugated estrogens + MPA (continuous)	0.625mg+ 2.5mg	O O	3	↑
	Conjugated estrogens + MPA (continuous)	0.625mg+ 5mg	O O	3	→
Post	Estradiol	50µg	T	4–13	→
2002	Estradiol	1mg	O	4–13	↑
	Estradiol + Gestodene (continuous)	1mg+25µg	O O	4–13	→
Decensi	Estradiol + MPA (12days/monthly)	50µg/d 10mg	T O	6–12	↑/→
2002	Conjugated estrogens + MPA (12days/monthly)	0.625mg 10mg	O O	6–12	↑
Skouby	Estradiol +	2mg	O	6–12	
2002	Cyproterone acetate (10/28 days) or	1mg	O		→
	Cyproterone acetate (10/21 days) or	1mg	O		→
	NETA (continuous) or	1mg	O		↑
	Levonorgestrel locally (spiral) or	20µg/24h	L		↑
	Medroxyprogesterone acetate (14/91 days)	20mg	O		↑ / →
Silvestri	Conjugated estrogens +	0.625 mg	O	3–6	↑
2003	MPA (continuous)	2.5mg	O		
Strandberg	Estradiol 2mg (12 days) + Estradiol 2mg + NETA 1mg (10 days) + Estradiol 1mg (6 days)		O	6	↑
2003	Estradiol + NETA (14days/monthly)	50µg 0.25mg	T T	6	→
Ylikorkala	Estradiol +	2mg	O	6–12	↑
2002	NETA (continuous)	1mg	O		

**O=oral; T=transdermal treatment, L=local, spiral in the uterus
MPA=medroxyprogesterone acetate
NETA= noretisterone acetate
↑ = rise
→ = no change
↑ / → = rise during estrogen phase, but decrease during MPA phase

AIMS OF THE STUDY

The present studies were aimed to clarify

1. the genetic background of intrahepatic cholestasis of pregnancy (ICP) in Finnish families

2. the incidence and risk factors for developing ICP

3. the effect of oral or transdermal postmenopausal hormone therapy on SHBG in women with a history of ICP

4. the effect of oral or transdermal postmenopausal hormone therapy on CRP in women with a history of ICP

5. liver and biliary diseases associated with ICP

SUBJECTS AND METHODS

The studies were conducted with the approval of the Ethics Committee of the Department of Obstetrics and Gynecology of Helsinki University Central Hospital in 1992–2004. Informed consent was obtained from the patients and controls in studies I, III and IV after explaining the purpose, nature and possible risks of the study. Studies II and V were register-based epidemiological studies, which were conducted in collaboration with the National Research and Development Centre for Welfare and Health in Helsinki.

1. Subjects

Sixty-nine pregnant women fulfilling the established criteria for ICP attended the Department of Obstetrics and Gynecology in Helsinki University Central Hospital during the years 1992–1993 (Study I) (Table 4). They were interviewed for a family history of ICP. The clinical features were compared in

Table 4. Number of patients and controls in each separate study.

Patients, controls by study	Time period	Number of subjects
Study I		
a) Women with intrahepatic cholestasis of pregnancy	1992–1993	69
b) All delivered women	1992–1993	5 304
Study II		
a) Women with a history of intrahepatic cholestasis of pregnancy	1987–2004	8 380
b) All delivered women	1987–2004	1 080 310
Study III, IV		
a) Women with a history of intrahepatic cholestasis of pregnancy	2000–2001	20
b) Control women	2000–2001	20
Study V		
a) Women with a history of intrahepatic cholestasis of pregnancy	1972–2000	10 504
b) Control women	1972–2000	10 504

The data for women in studies II and V were extracted from the Finnish Hospital Discharge Register. In studies I, III and IV women were collected from the patient register of the Department of Obstetrics and Gynecology Helsinki University Central Hospital.

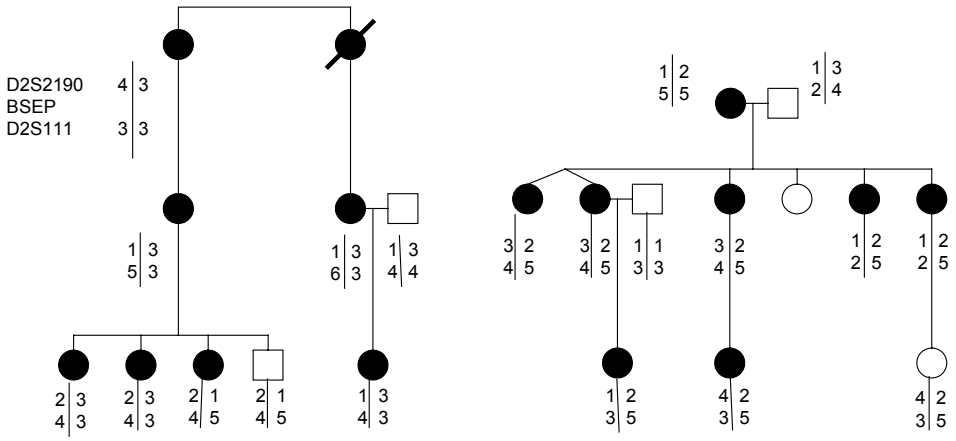


Figure 5. Two Finnish families with intrahepatic cholestasis of pregnancy in three pedigrees. The flanking markers for bile salt export pump (BSEP) in chromsome 2q24 are shown and respective alleles in genotyped individuals.

patients with a verified family history of ICP (n=11) and in patients with no such history (n=58).

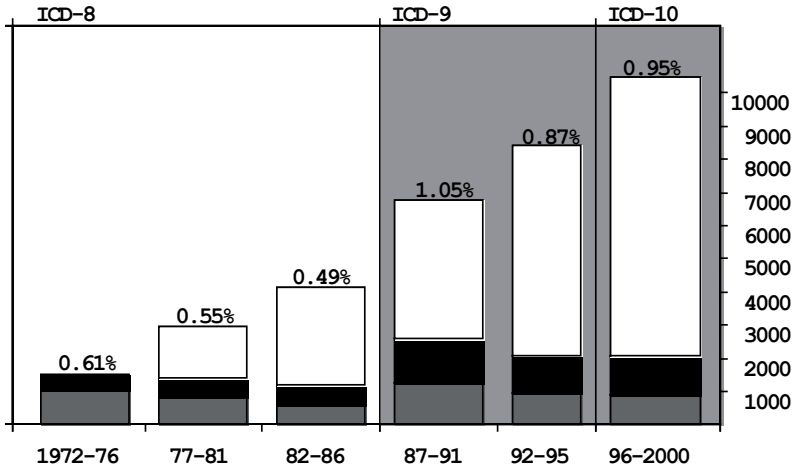
For the genetic analysis, 21 individuals (15 affected with ICP and 6 unaffected) from two independently ascertained families were genotyped. In these two families ICP had occurred in three consecutive generations (Figure 5) (Study I).

Data from women with ICP (n=8380) were collected from the Finnish Hospital Discharge Register in years 1987–2004 (Study II). During the study years two versions of the International Classification of Diseases, ICD-9 and ICD-10, were used and all women with the diagnosis numbers 6467A (n=4289) or 026.6 (n=3851) were enrolled. A proportion of women (n=240) had undergone their first ICP pregnancy before 1987, but they had also experienced a pregnancy complicated with ICP during the study years.

To study the effect of postmenopausal HT on liver function we invited 20 healthy postmenopausal women with a history of ICP (age 53-64 years) and 20 age-matched controls to participate in study (Study III, IV). They had entered menopause approximately six years before the study. All ICP women had a history of at least one pregnancy complicated by ICP and 13 of them had experienced ICP repeatedly. On average, ICP had occurred 30 years (26–33 years) before the study.

To evaluate the associations of liver and biliary diseases with ICP, we collected data from 10 504 women with a history of ICP and the same number of matched controls with no such history from the Finnish Hospital Discharge Register during the years 1987–2000 (Study V). Altogether 21008 women were included. Cumulative numbers of ICP women during these years are shown in Figure 6.

Figure 6. Cumulative numbers of women with intrahepatic cholestasis of pregnancy (ICP) during years 1972–2000 and during different International Classification of Diseases ICD-8, ICD-9 and ICD-10. Incidence of ICP for each time period is shown above the column.



Dark grey column: new ICP patient, age < 30 years
Black column: new ICP patient, age ≥ 30
White column: cumulative ratio of women, ICP diagnosed during earlier years
Light grey background: follow-up time during ICD-9 and-10

2. Methods

2.1.1. Linkage analysis

The genetic mapping of a disease with linkage analysis is based on the proximity of the disease locus to other previously known loci (marker loci) on the same chromosome. These loci are said to be linked. During meiosis each chromosome replicates into two sister chromatids. Homologous parental chromosomes are then paired before division occurs. During this synapsis crossing over (or recombination) may happen. As a result, exchange of chromosome material between the two homologous parental chromosomes occurs. The likelihood of a crossing over occurring between two loci is dependent on the physical and genetic distance between the two, the likelihood being higher the greater the distance. Genetic distances are expressed as centiMorgans (cM). When two loci are 1cM apart, there is a 1% chance of recombination between these two loci per meiosis. One cM contains about one million base pairs in physical distance. When the disease locus is linked to a marker, whose inheritance can be studied in a family, the location of the disease gene can be deduced by studying the affected and unaffected individuals in the family. Linkage analysis tests the likelihood that the disease locus and the marker locus are inherited together by proximity

against the likelihood that they are inherited by chance. This is expressed by the logarithm of odds, or LOD, score. In different families the LOD scores are calculated independently and can be combined. The power of the linkage analysis is stronger the larger the number of families with affected individuals, although many other factors also influence the power of the analysis.

The same principal can be utilized to test the association of a known gene with a specific disease. In those cases markers flanking the candidate locus or intragenic polymorphisms are used.

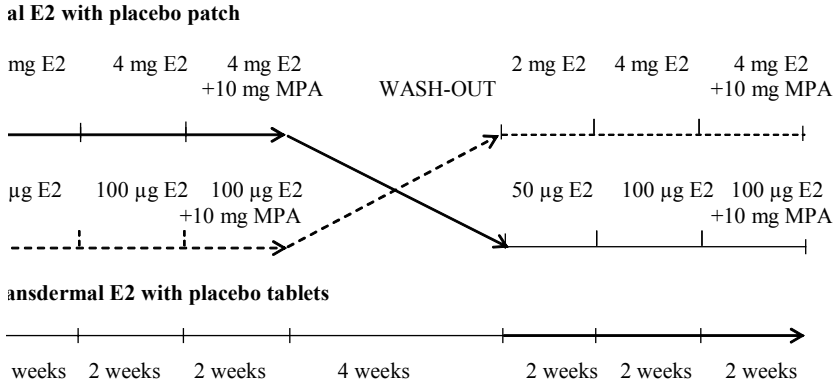
2.1.2. Polymerase chain reaction

Polymerase chain reactions (PCR) with primers for markers on chromosomes 2, 7 and 18 flanking the BSEP, MDR3 and FIC1 loci, respectively, were performed (Study I). The primer sequences were obtained from the GDB Human Genome Database. Buffers and AmpliTaq-Gold polymerase from Applied Biosystems (Roche, Branchburg, New Jersey, USA) were used during PCR.

2.2. Hormone therapy protocol

The women were randomized to receive increasing doses of estradiol (E2) orally (2mg for 14 days and 4mg for 14 days of estradiol valerate, Progynova ®, Schering AG, Berlin, Germany) or transdermally from a patch (50µg/24h for 14 days and 100µg/24h for 14 days of estradiol hemihydrate, FemSeven ®, Merck KgaA, Darmstadt, Germany) (Figure 7). The study was double-blinded in that during both treatments each woman took tablets (active or placebo) and used patches (placebo or active). At the end of the latter E2 period, medroxy-

Figure 7. Oral and transdermal estradiol followed by addition of MPA were given in 6-week periods with placebo patches or placebo tablets, respectively. The subjects were randomized to groups A (first treatment oral, solid line) and B (first treatment transdermal, hatsched line).



progesterone acetate (MPA) (10mg) (Lutopolar ®, Orion Pharma, Espoo, Finland) was given for 14 days concomitantly with E2. After a four-week wash-out period, the subjects were crossed over to the other treatment. Blood samples for alanine (ALAT) and aspartate (ASAT) transaminases, bile acids, GT, SHBG, CRP, E1 and E2 were drawn after an overnight fast and serum separated with centrifugation was stored –20°C until analyzed.

2.3. Assays

The levels of liver transaminases and bile acids were measured using auto-analyzers. Estrone (E1), E2, SHBG and CRP were assayed with established methods, the details of which are given in Table 5.

Table 5. Characteristics of the assays used.

Factor	Principle of assay	Source of reacent	Intra-assay CV	Inter-assay CV
Estrone	Radioimmunoassay (RIA)	Estron, Diagnostic Systems Laboratories, Sinsheim, Germany	<8%	<10%
Estradioli	Time-resolved fluoroimmunoassay (FIA)	Delfia, Wallac, Turku, Finland	<10%	<10%
SHBG	Time-resolved fluoroimmunoassay (FIA)	AutoDelfia, Wallac, Turku, Finland	<5%	<3%
CRP	Sensitive CRP Immunoentzymometric assay (IEMA)	Medix Biochemica, Kauniainen, Finland	<5%	<5%

CV=Coefficient of variation, SHBG=sex hormone-binding globulin, CRP=C-reactive protein

3. Statistical analyses

The incidences of ICP were age-adjusted using the direct-standardization technique and parous women during 1987–2004 as the standard population. Poisson regression analysis was used for the calculation of relative risks (RR). Rates, rate ratios and confidence intervals were calculated using standard techniques (Study II, V). Because the analyses incorporated significance tests for multiple but equally important endpoints, the p-values were Bonferroni-adjusted in order to detect any possible bias. The software packages Survo MM (www.survo.fi) and SAS v9.1 (www.sas.com) were used in the data processing and analysis.

In the crossover design (Study III, IV), the possibility of a period effect was tested by the Mann-Whitney test, where we compared the differences between the periods in the two groups of patients (those beginning with oral and those beginning with transdermal E2); no period effect was detected. The possibility

of a treatment-period interaction was investigated by the Mann-Whitney test, which compared the average responses to the two treatments. They were unrelated to the order of treatment. Therefore, women using HT orally or transdermally were analyzed as separate groups. Because of the skewed distribution of SHBG, CRP, E2 and E1 data, non-parametric tests (Wilcoxon's signed-rank test) were used for comparison. Non-parametric repeated measures ANOVA (Friedman test) were used to test variation among groups during treatment and Wilcoxon's signed-rank test was used to test changes between the groups. Correlation analyses were performed using Spearman's non-parametric correlation coefficient. A two-tailed $p<0.05$ was accepted as the level of significance.

RESULTS

Detailed results are given in the original publications and therefore, only the main results are summarized here.

1. Genetics

Positive family history for ICP was detected in 16% (11/69) of women. The levels of serum transaminases were higher in the familial than in the sporadic group (Table 6). Also the recurrence rate of ICP in familial cases (92%) was higher ($p<0.05$) as compared to that in sporadic cases (40%). ICP complicated 14% and 43% of twin and triplet pregnancies, respectively.

Table 6. Two highest alanine (ALAT) and aspartate (ASAT) aminotransferase and bile acid values (mean; SEM), and comparison of clinical features of intrahepatic cholestasis of pregnancy (ICP) between familial and sporadic cases (number; %).

	Normal range	Familial (n=11)	Sporadic (n=58)
ASAT (U/l)	15–35	265.0 (66.0)	106.0 (9.0)**
ALAT (U/l)	10–40	348.7 (85.5)	190.5 (22.3)**
Bile acid (μmol/l)	<6	29.4 (4.8)	32.0 (4.4)
Number of multiple pregnancies	149/5304 (2.8%)	3/11 (27%)	22/58 (38%)
Occurrence of ICP in previous pregnancies		11/12 (92%)	18/45 (40%)**

**$p<0.05$

Three known cholestasis genes were studied with markers flanking each locus. Amplification of microsatellite markers on chromosomes 2 (D2S2190, D2S111), 7 (D7S644, D7S2410) and 18 (D18S977, D18S849) flanking the BSEP, MDR3 and FIC1 locus, respectively, was performed with polymerase chain reactions (PCR) (Study I). The distance between the markers D2S2190 and D2S111 at the gene locus *ABCB11* coding for the bile salt export pump is 4.8cM. Multipoint linkage analysis gave positive LOD scores, maximum 1.12, over this region in family 2, but negative scores in family 1. Even though LOD score was positive it did not mean that the genes are linked. In haplotype analysis (Figure 5), the affected individuals in family 1 had different alleles, suggesting that mutations in this region are not associated with ICP in these women. The linkage analysis of the *ABCB4* gene coding for MDR3 and the *ATP8B1*

gene coding for FIC1 gave negative LOD scores in both families. Additionally, two members from each of the two large families were screened for mutations in the *ABCB4* gene by sequencing, but none were found.

2. Incidence and risk factors

The incidence of ICP in deliveries in Helsinki University Central Hospital (1.3%) (Study I) was slightly higher than that in the nation-wide study (1.0%) (Study II) and did not show any significant changes during 1987–2004. For the register-based study, the crude and the age-adjusted incidences and the numbers of women with ICP during three 6-year periods are shown in Table 7.
The risk of ICP correlated with rising maternal age. The risk in women > 39 years of age was 3-fold higher than that in women < 30 years when adjusted for parity and multiple pregnancies (RR 2.98; 95% CI 2.73–3.25). The risk of ICP was also associated with maternal parity being higher in nulliparous compared to multiparous women (incidence 1.24% versus 0.80%). Likewise, the risk of ICP was higher in multiple versus singleton pregnancies (RR 4.54; CI 4.19–4.91). Multiple pregnancy was an independent risk factor, whereas there was a significant interaction between maternal age and parity. Using parous women < 30 years of age as the reference group, the RR of ICP for nulliparous women was 1.34 (CI 1.26–1.42) in women < 30 years, 2.41 (CI 2.26–2.58) in women 30-39 years, and 5.52 (CI 4.73–6.44) in women > 39 years of age. The incidence was highest 16.9 % (CI 13.6–20.2) in the first, multiple pregnancies of women > 39 years.

Table 7. The crude and age-adjusted incidences of ICP and number of pregnancies with ICP (n) during three 6-year period 1987–2004.

Periods	1987–1992	1993–1998	1999–2004
Crude incidence	0.95 %	0.87 %	1.02 %
Age-adjusted incidence	0.98 %	0.87 %	1.00 %
Pregnancy	n	n	n
Singleton, nulliparous women	1 571	1 222	1 362
Singleton, parous women	1 835	1 701	1 818
Multiple pregnancy, nulliparous women	116	131	120
Multiple pregnancy, parous women	87	116	106
Total number of ICP pregnancies	3 609	3 170	3 406

3. The effect of hormone therapy on sex hormone-binding globulin

Oral and transdermal E2 administration was not accompanied by significant changes in levels of liver transaminases or bile acids. The levels fluctuated but remained within the normal range in each subject.

Oral E2 therapy increased liver SHBG production in women, with and without a history of ICP, after two weeks of treatment (Table 8). In the ICP group, SHBG concentrations increased during 2mg and 4mg doses of E2 daily by 42% and 121% and in control women by 67% and 171%, respectively. The response of SHBG to increasing E2 was blunted in the ICP group (median rise SHBG 38.2 nmol/l) compared to controls (median 59.4 nmol/l) ($p=0.006$). The addition of MPA was associated with significant decreases in SHBG levels both in ICP (14%) and control women (16%). In the control group, the SHBG responses correlated positively with E2 during weeks 0–2 ($r=0.66$, $p=0.002$), weeks 0–4 ($r=0.49$, $p=0.03$) and weeks 0–6 ($r=0.51$, $p=0.02$) whereas in ICP women such a correlation existed only during weeks 0-4 ($r=0.63$, $p=0.003$). Increases in E2 concentrations did not differ between the ICP and control women.

Transdermal E2 with higher dose (100μg/day) raised SHBG concentration only in the control group (Table 9). Addition of MPA orally to the transdermal regimen reduced SHBG production in both groups.

Table 8. Responses from baseline of sex hormone-binding globulin (SHBG) (nmol/l) and C-reactive protein (CRP) (mg/l) (median, 95% confidence interval) to oral estradiol (E2) therapy alone and in combination with medroxyprogesterone acetate (MPA) (10mg/day) in women with a history of intrahepatic cholestasis of pregnancy (ICP) and control women.

	E2	dose/day	Δ p	E2 + MPA	Δ p
	2 weeks 2mg	+2weeks 4mg	weeks 0–2 vs weeks 0-4	+2weeks4mg +MPA 10mg	weeks 0–4 vs weeks 0-6
SHBG					
ICP (n=20)	25 (10–38)	62 (48–106)	p<0.001	66 (34–71)	p=0.010
Control (n=19)	30 (24–56)	81 (57–103)	p<0.001	57 (44–90)	p<0.001
CRP					
ICP (n=20)	0.2 (0.1–1.1)	0.6 (0.3–1.6)	p=0.020	0.4 (0.2–1.1)	NS
Control (n=19)	0.4 (0.1–0.9)	0.7 (0.3–2.4)	p=0.020	0.8 (–0.3–1.3)	NS

Δ p= significance to the responses

Table 9. Responses from baseline of sex hormone-binding globulin (SHBG) (nmol/l) and C-reactive protein (CRP) (mg/l) (median, 95% confidence interval) to transdermal estradiol (E2) therapy alone and in combination with medroxyprogesterone acetate (MPA) (10mg/day) in women with a history of intrahepatic cholestasis of pregnancy (ICP) and control women.

	E2	dose/day	Δ p	E2 + MPA	Δ p
	2 weeks 50µg/day	+2 weeks +100µg/day	weeks 0–2 versus weeks 0–4	+2 weeks +100µg/day +MPA 10mg	weeks 0–4 versus weeks 0–6
SHBG					
ICP (n=20)	–0.4 (–3.4–3.9)	3.7 (–1.6–6.2)	NS	–6.6 (–17 – –2.0)	p=0.003
Control (n=19)	–1.5 (–8.9–4.7)	3.0 (–2.0–12)	p=0.020	–9.5 (–16 – – 2.3)	p<0.001
CRP					
ICP (n=20)	–0.2 (–0.8–0.2)	–0.02 (–0.9– 0.4)	NS	–0.2 (-0.4–0.4)	NS
Control (n=19)	–0.03 (–0.1–0.2)	0.02 (–0.2–0.9)	NS	–0.2 (–0.5–0.1)	NS

Δ p=significance to the responses

4. The effect of hormone therapy on C-reactive protein

The levels of CRP rose within two weeks during oral E2 use. There were no differences in CRP rise during oral 2mg and 4mg estradiol dose between women with and without a history of ICP. When both women with and without ICP history were analyzed together the rise in CRP was dose-dependent (49% and 91%) (Table 8) and the changes in serum concentrations of CRP and E2 correlated positively (r=0.326, p=0.007). The administration of MPA during the last two weeks with higher E2 dose had no effect further on CRP production (which was already stimulated by oral E2).

Transdermal E2 had no effect on the CRP production in either group although the dose was increased to 100μg/day. The administration of MPA to transdermal regimen had no effect on CRP (Table 9).

5. Associated liver and biliary diseases

Several liver and biliary diseases were associated with a history of ICP (Table 10). The rate ratio for non-alcoholic cirrhosis with liver fibrosis was 8.2 (CI 1.9–35.5) and for PBC alone 5.8 (CI 0.7–48). There were six cases of PBC in the ICP group, but none in the control group. A history of ICP was associated with hepatitis C, and with non-specific hepatitis (including autoimmune hepatitis).

The incidence of cholecystitis and cholelithiasis was almost fourfold higher in ICP women with rate ratio 3.7 (CI 3.2–4.2). In an age-group analysis it was clearly seen that women with ICP had gallstones at a younger age than controls. Non-alcoholic pancreatitis was more common in ICP patients than in controls (3.2, CI 1.7–5.7), whereas the rate ratio for alcohol-induced pancreatitis was not significantly different in patient and controls.

Table 10. Diseases of the liver and biliary system in women with a history of intrahepatic cholestasis of pregnancy (ICP) and in the control women. Rate ratio (cases/controls) and 95% confidence interval (CI) is shown.

Disease	**ICP (n=10 504)**	**Control (n=10 504)**	**Rate/ ratio**	**95% CI**	**p**
Hepatitis C and non-A-non-B	29	8	3.5	1.6–7.6	<0.001
Hepatitis, non-specific	26	6	4.2	1.7–10.2	<0.001
Cirrhosis, non-alcoholic	17	2	8.2	1.9–35.5	<0.050
Cholelithiasis and cholecystitis	965	260	3.7	3.2–4.2	<0.001
Biliary system disorders	45	15	2.9	1.6–5.2	<0.001
Non-alcoholic pancreatitis	46	14	3.2	1.7–5.7	<0.001

DISCUSSION

Intrahepatic cholestasis of pregnancy (ICP) affects approximately 1% of pregnancies in Finland (Laatikainen et al 1984), and is probably the most common intrahepatic cholestatic condition world-wide. The relative hepatic incapacity to metabolize large amounts of pregnancy-related steroids is one of the major contributing factors (Reyes 1993), but still the exact etiology has remained unknown. It has been noted that ICP occurs in families suggesting genetic susceptibility (Holzbach et al 1983, Hirvioja et al 1993). Recently, the genetic backgrounds of several types of progressive familial cholestasis were identified (Jacquemin 2001). This has raised interest in the possible molecular genetic cause of ICP as well.

In a genetic isolate the frequency of certain genetic variants and mutations may be high due to geographical enrichment. In Finland this has been shown both for alleles predisposing to common, complex diseases (Vuorio et al 2001) and mutations causing less common single gene disorders (Aittomäki et al 1995). As ICP may behave as a single gene disorder in some families but as a complex trait in most cases, it should be possible to study both of these situations in the Finnish population. To elucidate the clinical picture and heredity of ICP we studied a series of 69 patients from whom detailed family history of ICP was collected. To study the genetic background of ICP we performed molecular genetic studies to find the possible genes behind ICP in two Finnish families.

Moreover, we wanted to determine the incidence of ICP, possible risk factors and associated liver and biliary diseases in Finland. For that purpose the rather uniform diagnosis of ICP and high standard register gave an excellent opportunity. We collected patients and controls from the nation-wide data base, the Finnish Hospital Discharge Register. In register-based studies biases may arise from case-control collection and this may affect the results. To avoid this bias the controls and patients were matched for age as well as time and place of delivery. The personal identification number, which was used as a linkage key, excludes the possibility of a double enrollment of the same individual. The validity of the Finnish Hospital Discharge Register has been evaluated in several studies. Virtually all inpatient hospital care periods in Finland are recorded in the register because the reporting is compulsory. The accuracy of most variables utilised in this study (personal and hospital ID-numbers, age, sex, and dates of admission and discharge) is at least 95%. The recorded main diagnosis is typically consistent (about 95%) with the medical record and for the subsidiary diagnoses the consistency is also good. (Mäkikyrö et al 1998, Leppälä et al 1999, Gissler et al 2004, Pajunen et al 2005).

Finally, we studied the effects of oral and transdermal HT on the production of SHBG and CRP. Liver dysfunction, even if subclinical, may have an effect on the protein production. The study design, a double-blind prospective cross-over study on 40 postmenopausal women, enabled the true comparison of the effects of oral and transdermal HT on SHBG and CRP in women with and without a history of ICP.

Incidence, risk factors and genetic background of ICP

The incidence of ICP was 1.1% in the Helsinki University Central Hospital in the 1970's and 1980's (Laatikainen et al 1984). In our hospital-based series the incidence of ICP was 1.3% in the 1992. A somewhat lower incidence (0.54%) was reported from Kuopio University Hospital in 1990 (Heinonen et al 1999) but that study had excluded all multiple pregnancies, which predispose to this disease. In the present nationwide study ICP occurred in 0.94% of pregnancies and this rate did not change markedly during 1987-2004. An unchanged incidence of ICP supports the idea that genetic predisposition could be one etiologic factor. However, as only inpatient care is recorded in our register the incidence of ICP we report is the minimum incidence. It is possible that some patients with ICP may have been treated as outpatients and were not included in our study. Moreover, some patients could be lost due to non-reporting of the diagnosis, because this risk appears higher for secondary diagnosis, while the first diagnosis is known to be more reliable (Sund 2003, Gissler et al 2004).

We found that multiple pregnancy predisposes to the development of ICP, as has been shown in small series (Laatikainen et al 1984, Gonzalez et al 1989, Glantz et al 2004). The hospital-based study showed a higher risk of ICP (14% of twin pregnancies) than the register-based study (4.0%).

As a novel finding we present strong evidence that advanced maternal age predisposes to ICP. The risk of ICP showed a positive correlation with maternal age and is 3-fold higher in women over 39 years of age than in those under 30 years. The risk was further enhanced by nulliparity. These findings may explain, at least in part, why an elevated risk of ICP (RR 3.8) has been found in in-vitro-fertilization (IVF) pregnancies (Koivurova et al 2002). Women undergoing IVF are typically older, nulliparous women.

We demonstrate that ICP is familial in 16% and sporadic in 84% of cases. The background of ICP may actually differ between these two groups of patients, and this is also suggested by the differences in the clinical features such as significantly higher levels of transaminases and higher rate of recurrence in familial cases. Recently the genetic backgrounds of several types of cholestasis, including PFIC types 1, 2, and 3 with mutations in *ATP8B1, ABCC11,* and *ABCB4*, respectively, have been identified. The possible association of ICP with some of these genes was initially suspected, because same heterozygous mothers of children affected with PFIC3 had experienced ICP during pregnancy

(Jacquemin 2001). Although autosomal dominant inheritance has been suggested, it is uncommon to find large families with many females affected with ICP. We were able to study two such families in whom ICP had occurred in at least 15 individuals in 3 generations (Figure 6). However, none of the above mentioned cholestasis genes were found to be implicated in ICP etiology in our study.

Hormone use and liver proteins

Oral and transdermal HT was well tolerated during this short study with increasing doses of E2. Liver transaminase activities remained within normal ranges in women with a history of ICP and controls. This can be seen as evidence that the liver can metabolize both oral and transdermal E2 normally in women with a history of ICP.

Postmenopausal oral HT has shown to increase the production of SHBG (Stomati et al 1996, Serin et al 2001, Samsioe 2002). Women with a history of ICP were characterized as having normal levels of SHBG before the initiation of HT. The levels of SHBG rose with increasing doses of E2 in both women with and without a history of ICP. This must be a reflection of the stimulated synthesis of this protein in the liver. Compared to control women, the response of SHBG to oral E2 was blunted in ICP women. Because no data imply that ICP should affect the degradation of SHBG, our data suggest that it is an inborn feature of these women to have a suppressed response of SHBG to oral estrogen. In theory, these women may therefore become exposed to higher levels of free estrogen during the use of oral E2, and this effect may have both positive (e.g. bone) and negative (e.g. breast, gallstones and cardiovascular) effects. As transdermal E2 does not stimulate the synthesis of SHBG the route of estrogen administration may have an important role. The higher dose of transdermal E2 and oral MPA induced decreases in SHBG concentrations. This may be mediated in part through a rise in insulin-like growth factor 1 (IGF-1), which has been shown to decrease SHBG production (Kalme et al 2003). High doses of MPA have been shown to increase the production of IGF-1 (Saaresranta et al 2002).

Long-term oral postmenopausal HT has been shown to be associated with elevated levels of CRP (van Baal et al 1999, Post et al 2002, Ylikorkala et al 2002, Silvestri et al 2003). This rise may account for the lack of effect of HT in primary (Manson et al 2003) and secondary (Hulley et al 1998) prevention of ischemic cardiac events.

Oral E2 raised the serum concentrations of CRP within two weeks both in women with a history of ICP and controls, whereas transdermal HT had no effect. This is in accordance with previous studies (van Baal et al 1999, Post et al 2002, Ylikorkala et al 2002, Silvestri et al 2003). We could demonstrate that the rise in CRP was E2 dose-dependent, although it is possible that some of this ef-

fect could be due to a prolonged effect of the preceding 2mg treatment as previously suggested (van Baal et al 1999). The increases in CRP concentrations were related to the rises in E2 only during oral use. Our data also show that the effect of oral E2 on CRP vanishes totally in four weeks.This supports the role of the liver in estrogen-induced elevation of CRP concentrations. We did not study the effect of HT on IL-6, which is suggested to be the primary stimulating factor for CRP synthesis in inflammatory reactions (Straub et al 2000), but it has been shown that this cytokine is not increased during oral HT use (Lakoski et al 2005). It is also known that adiposity is correlated with higher CRP levels without HT (Barinas-Mirchell et al 2001). Obese postmenopausal women, due to higher aromatization in fat tissue (Ruggiero et al 2002, Tapanainen 2003), have higher E1 concentrations and this might be one explanation for CRP elevation in obese women. On the other hand, both human intra-abdominal and subcutaneous abdominal fat is known to produce IL-6 (Fried et al 1998). Of course we must take into account that the highest transdermal dose was 100μg/day, and we do not know if higher doses of E2 would have stimulated CRP production.

It has also been thought that the rise in CRP during HT could be a sign of a proinflammatory effect of estrogen which may accelerate atherosclerosis and vascular occlusion (Lagrand et al 1999), yet most recent data do not uniformly support this hypothesis (Lakoski et al 2005). Anyway, our data can be seen as evidence that women with a history of ICP can use HT safely, both orally and transdermally, at least as it concerns liver function.

Consequences of ICP on liver and biliary tract

No later liver consequences, except for the risk for cholelithiasis, have been established for ICP and ICP has been considered to be specific to the pregnancy. Our study did not support this. Cirrhosis was more common in women with a history of ICP. Of course we could not exclude with full certainty the possibility that these liver diseases could have preceded the onset of ICP subclinically, because these diseases may exist in a mild form and remain undiagnosed during pregnancy. We also found increased incidence of hepatitis C infection in ICP patients than in the controls, while there was no difference in the risk of A and B hepatitis. This was in line with the only previous study from Italy (Locatelli et al 1999), although the diagnostic criteria for ICP in the Italian study were not entirely similar to ours. It has been suggested that the hepatitis C virus could down-regulate the expression of the transporter MRP2 and this could cause the hepatocyte damage (Hinoshita et al 2001). The situation may resemble that in Dubin-Johnson syndrome, which is caused by mutations in the *MRP2* gene. Like ICP patients, patients with Dubin-Johnson syndrome may develop jaundice during the intake of high-dose oral contraceptives. (Pauli-Magnus et al 2005). A similar mechanism could trigger ICP, although we could

not demonstrate a connection between ICP and diseases of bilirubin metabolism in our study.

We could also confirm that the risk of cholelithiasis and cholecystitis was elevated in women with a history of ICP. Mutations in the genes coding proteins MDR3 and BSEP have been associated to cholelithiasis (Jacquemin 2001, Rosmorduc et al 2003, van Mil et al 2005) We regard this higher rate as a true consequence of ICP, although a more frequent use of ultrasound examination in women with ICP during pregnancy may cause bias: asymptomatic patients with gallstones become more often detected in ICP group. It was also shown that women with ICP develop gallstones at a much younger age than controls. Both endogeneous and exogeneous estrogens and perhaps progestins may have a role in this (Dowling 2000, Simon et al 2001, Cirillo et al 2005). Gallstones and alcohol abuse are the most important causes of pancreatitis (van Brummelen 2003). Cholelithiasis may explain the higher frequency of pancreatitis (not alcohol-induced). However, the *ATP8B1* gene is strongly expressed in extrahepatic tissues such as pancreas, small intestine and kidney (Pauli-Magnus et al 2005). The presence of diarrhea and pancreatitis in patients with PFIC1 suggest a role in extrahepatic tissues. Finally, a genetic factor connecting cholelithiasis with ICP are mutations in the *ABCB4* gene (coding MDR3), which have been found in patients with gallstones and in patients with ICP (Jacquemin et al 2001, Rosmorduc et al 2001). To resolve the causes of associations between ICP and these diseases, further studies are clearly needed.

CONCLUSIONS

1. ICP was familial in at least 15% of patients and in some families showed autosomal dominant inheritance. The disease mechanisms in sporadic and familial cases might not be entirely similar as there were differences in the phenotypes between the two groups. Mutations in three genes (*ABCB4*, *ABCB11* and *ATP8B1*) regulating bile acid transport were not encountered in two Finnish families with ICP.

2. The incidence of ICP in Helsinki University Central Hospital in 1992–1993 was 1.3% and ICP recurred in 92% of familial and in 40% of sporadic cases. In a nationwide study the incidence of ICP was slightly smaller (1.0%) and remained stable during the years 1987–2004.

3. Maternal age, parity, and multiple pregnancy were all risk factors for ICP. The recognition of these allows identification of special risk pregnancies.

4. The livers of women with a history of ICP tolerated well the increasing doses of estradiol, both orally and transdermally, although the response of SHBG was slightly blunted during the oral regimen. Likewise, the liver of these women could increase the synthesis of CRP in a dose-dependent manner in response to oral estradiol already within two weeks; this response vanished in four weeks after the cessation of treatment. The activities of liver transaminases remained within the normal range. Transdermal estradiol had no effect on liver CRP or SHBG.

5. Women with a history of ICP were at a higher risk of some other liver and biliary diseases including non-alcoholic cirrhosis, non-specific hepatitis, hepatitis C, cholelithiasis and cholecystitis and pancreatitis in their later life.

6. Clinicians should inquire as to the history of ICP because it may have an influence on the health of a woman. Further studies are needed to resolve whether the women at risk of subsequent liver diseases could be identified already during an ICP pregnancy.

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Anne Leppänen

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