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Progress and Prospects in Gastroenterology

Current progress in our understanding of cholestasis and chronic cholestatic disorders

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Cholestasis results from any cause that impairs bile acid transport from the liver to the intestine. The formation of bile as well as the biliary secretion of many endogenous and exogenous substances are essential functions of the liver. The secretion of bile depends on the function of several transporters found on the membranes of hepatocytes and cholangiocytes. Many of these transporters have now been cloned, several molecular defects have been identified and associated with various forms of cholestatic diseases. At the histological level, chronic cholestastic diseases result from inflammatory and destructive processes that may involve the intrahepatic and/or extrahepatic biliary tree or from developmental defects. Most chronic cholestatic diseases may progress towards biliary cirrhosis, portal hypertension, and eventually hepatocellular insufficiency. Chronic cholestatic conditions can be separated into those presented mainly in infancy and childhood and those presented first in adulthood (Table 1). This review summarizes some of the recent developments in our understanding of the hepatobiliary transport as well as of the pathogenesis and classification of chronic cholestatic disorders.

HEPATOBILIARY TRANSPORT

The hepatobiliary transport certainly starts from the hepatocytes. Hepatocyte transport processes are responsible for the formation of primary bile and for the major part of the excretory function of the liver, while cholangiocellular transport processes are mainly responsible for dilution, modification, and alkalization of the primary bile. Hepatocytes express transporters at both the basolateral (sinusoidal) and apical (canalicular) membrane domains that collaborate to serve the transport of substances from the blood into bile[1,2] (Figure 1).

The transporters of the basolateral membrane that mediate the uptake of bile salts and other organic anions can be divided into Na^+-dependent and Na^+-independent systems. The Na^+-dependent transport systems are responsible for more than 80% of the hepatocellular uptake of conjugated bile salts and for less than 50% of cholate uptake. The main transport system in this group is the Na^+-taurocholate cotransporting polypeptide (NTCP), a 349-amino acid protein, that is structurally related to the intestinal bile salt transporter. The Na^+-independent hepatocellular uptake of bile acids is mediated by a family of transport proteins, called the organic anion transporting polypeptides (OATP). The first OATP identified in human liver (OATP-A) mediates basolateral transport of bile acids, organic anions such as bromosulphophthalein, conjugated steroids, type II cations, and several drugs. The OATP-A gene has been localized to chromosome 12p12. Two additional proteins of the human OATP family have been identified: the OATP-B, a prostaglandin transporter (PGT), and the OATP-C, a liver-specific transporter (LST-1) that transports conjugated steroids, eicosanoids, thyroid hormones and taurocholate. The OATPs play a central role in hepatocellular organic anion and drug clearance. Several ATP-dependent pumps from the family of multidrug resistance proteins (MRP1, 3, 5, 6) have also been isolated at the basolateral membrane. MRP3 mediates basolateral efflux of the organic anions estradiol-17β-D-glucoronide and glutathione, of bile salts, and of the drugs methotrexate and etoposide. Other classes of basolateral transport proteins are the organic cation transporters (OCT) family and the organic anion transporters (OAT)

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Table 1. Chronic cholestatic diseases

In infancy and childhood	In adulthood
Biliary atresia	Primary biliary cirrhosis
Syndromic bile duct paucity (Alagille syndrome)	Primary sclerosing cholangitis
Non-syndromic bile duct paucity	Drug induced cholestasis
Infection	Benign recurrent intrahepatic cholestasis
Chromosomal disorders	Idiopathic adulthood ductopenia
Metabolic	Lymphoma
Inborn errors in bile acid synthesis	Infiltrative, granulomatous diseases
	Amyloidosis
	Sarcoidosis
	Tuberculosis
	Malignancy
	Bile duct stenosis, strictures
	Allograft rejection
	Graft versus host disease
	Parenteral nutrition
	Stauffer syndrome

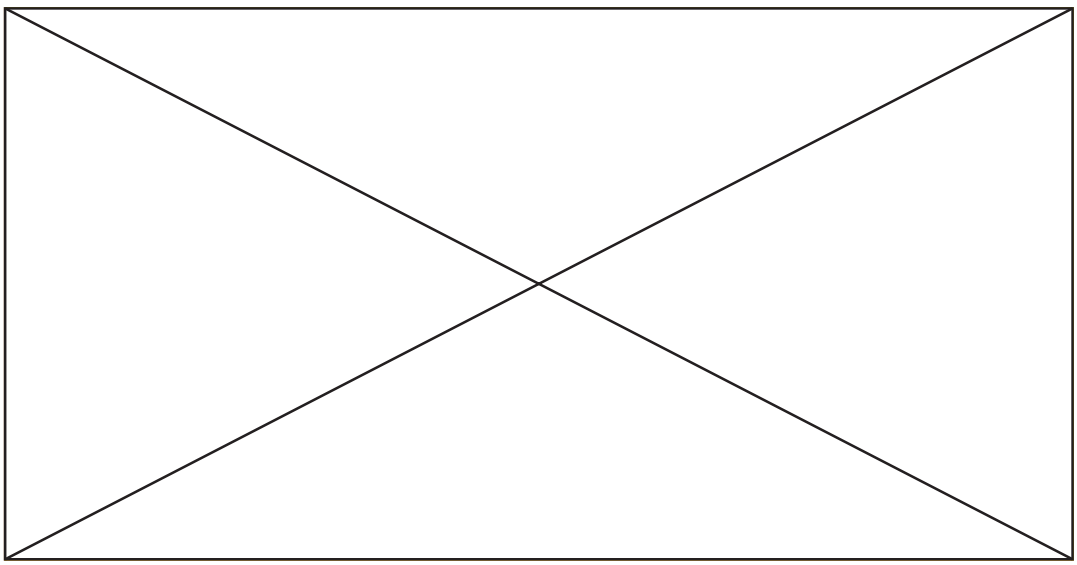

Figure 1. Hepatobiliary transporters. Bile salts inflow from blood into the hepatocytes is mediated mainly by the Na^+-taurocholate cotransporting polypeptide (NTCP) and to a lesser degree by the organic anion transporting polypeptides (OATP), which consist of a Na^+-independent uptake system. NTCP and OATPs are also involved in the clearance of other substances, mainly organic anions and drugs. The role of other classes of basolateral transport proteins, such as the organic cation transporters (OCT) family and the organic anion transporters (OAT) family remains unclear. The multidrug resistance proteins (MRP1, 3, 6), which are ATP-dependent pumps, are mainly responsible for the basolateral efflux of organic anions, including bile salts and drugs. The bile salt export pump (BSEP), an ATP-dependent pump, is mainly responsible for the canalicular secretion of bile salts, while the secretion of non-bile salt organic anions, such as conjugated bilirubin, into bile is mediated by the canalicular multidrug resistance protein 2 (MRP2). A class III multidrug resistance P-glycoprotein (MDR3), an ATP-dependent flippase, is responsible for the canalicular secretion of phospholipids, mainly phosphatidylcholine and alkalization of bile (chloride with bicarbonate exchange) is mainly achieved by the anion exchanger 2 (AE2). Finally, the multidrug resistance gene product MDR1 is involved in the canalicular transport of cytotoxic cations.

family; the latter has not been isolated in humans. The exact role of these transporters remains unclear.

Transport across the canalicular membrane is the rate limiting step for the biliary secretion and formation of bile. The canalicular secretion of bile salts is predominantly mediated by an ATP-dependent pump, called "bile salt export pump" (BSEP). Cholesterol and phospholipids are the other major lipids secreted in bile. No transport protein for cholesterol secretion has been identified to date, but there are suggestions that a pump of the superfamily of ATP-biding cassette (ABC) might be involved. On the other hand, an ATP-dependent flippase, a class III multidrug resistance P-glycoprotein (MDR3), has been found to be responsible for the secretion of phospholipids, mainly phosphatidylcholine. The secretion of non-bile salt organic anions, such as conjugated bilirubin, into bile is mediated by the canalicular multidrug resistance protein 2 (MRP2). Moreover, chloride with bicarbonate exchange is mediated by the anion exchanger 2 (AE2), while the multidrug resistance gene product MDR1 is involved in the transport of cytotoxic cations.

Finally, the primary bile is modified in the bile ducts by the cholangiocellular transport processes. Cholangiocytes express the AE2, a chloride/bicarbonate exchanger, and the cystic fibrosis transmembrane regulator (CFTR), which also represents a chloride channel. Both transporters are responsible for alkalization and bicarbonate enrichment of the primary bile. Moreover, conjugated bile salts are partially reabsorbed in the bile ducts via a Na^+-dependent bile salt transporter.

CHRONIC CHOLESTATIC DISEASES IN INFANCY AND CHILDHOOD

Anatomical abnormalities or malfunctions of the hepatobiliary transport proteins may be responsible for chronic cholestatic conditions in this age group (Table 1). It should be noted that isolated defects in specific transporters can be associated with jaundice but without frank cholestasis. In particular, the Dubin-Johnson syndrome, which is characterized by direct hyperbilirubinemia and pigment deposition in the liver, is an inherited disorder caused by the absence of the MRP2 protein due to *mutations in the MRP2 gene*[3]. Since the MRP2 protein is responsible for the non-organic anion transport (conjugated bilirubin) and the bile salt dependent formation of bile remains intact, no frank cholestasis develops.

BILIARY ATRESIA

Biliary atresia is still considered an *idiopathic* inflammatory process resulting in destruction of the lumen of the extrahepatic biliary tract and sometimes even of the intrahepatic bile ducts. Although it is the most frequent cause of chronic cholestasis and the most frequent indication for liver transplantation in infants and children, its etiology remains unknown. The hepatoportoenterostomy (Kasai procedure) remains the treatment of choice for early stages, while liver transplantation is the only therapeutic option for advanced stages of the disease.

SYNDROMIC PAUCITY OF INTRAHEPATIC BILE DUCTS (ALAGILLE SYNDROME)

Alagille syndrome is inherited by an autosomal dominant trait with variable expression and represents the most frequent form of familial intrahepatic cholestasis. It presents with persistent jaundice (direct hyperbilirubinemia) usually at 1-2 years of age, characteristic facial features, congenital heart disease, vertebral defects, and posterior embryotoxon. Several mutations in the human Jagged gene (chromosome 20) are responsible for this syndrome[4], but the type of genetic change has not been associated with specific phenotypic characteristics. Moreover, the mechanism(s) by which these mutations lead to the disease has not been clarified yet.

Non-syndromic paucity of intrahepatic bile ducts

Non-syndromic paucity of intrahepatic bile ducts may be caused by infection cytomegalovirus, rubella, syphilis), chromosomal disorders (trisomy 18, 21) and metabolic disorders [α_1-antitrypsin deficiency, cystic fibrosis, progressive familial intrahepatic cholestasis (PFIC)]. Recent studies have identified the genes responsible for cystic fibrosis and PFIC. Cystic fibrosis is caused by a hereditary defect of *CFTR*[5]. It has been shown that defective chloride channel activity impairs ductal bile salt secretion and may cause cholestasis. Cystic fibrosis may eventually cause cholestasis by several other mechanisms too. PFIC is a familial disorder inherited in an autosomal recessive fashion. PFIC comprises at least three distinct clinical entities[6]. All cases usually present with intrahepatic cholestasis within the first year of life and lead to cirrhosis and liver failure.

PFIC type 1 (PFIC1 or Byler disease) is characterized by recurrent episodes of jaundice, pruritus, high concentrations of serum bile salts, and normal gamma-glutamyl-transpeptidase (γGT) and cholesterol levels. Liver disease is progressive, with cholestasis but not duc-

tular proliferation, leading to death from liver failure within the first decade of life. PFIC1 is caused by mutations of the *PFIC1 gene* located on chromosome 18q 21-22. The PFIC1 gene is expressed in the liver and small intestine and encodes a *P-type ATPase* involved in the transport of aminophospholids from the outer to the inner leaflet of various membranes. Since the transport of bile salts is impaired not only in the liver but also in the intestine, the patients develop not only cholestasis but chronic watery diarrhea as well.

PFIC type 2 (PFIC2 or Byler syndrome) resembles PFIC1 but has a more severe initial presentation with permanent jaundice and rapid progression to liver failure. PFIC2 is caused by mutations of the *BSEP gene* located on chromosome 2q 24, which encodes for the *bile salt export pump* (*BSEP*) of the canalicular membrane. Thus, accumulation of bile salts within the hepatocytes due to impaired BSEP function causes progressively increasing hepatocellular injury due to apoptosis and/or necrosis.

PFIC type 3 (PFIC3 or MDR3 deficiency) is associated with an inflammatory infiltrate in the early stages, ductular proliferation and high γGT levels. However, liver injury may also progress to biliary cirrhosis and liver failure in such patients. PFIC3 is caused by homozygous mutations of the *MDR3 gene* and the subsequent lack of the MDR3 P-glycoprotein, which is responsible for the canalicular transport of phospholipids. Given the protective role of phospholipids for the biliary epithelium, their absence from bile in MDR3 deficiency is associated with liver injury due to unbalanced toxicity of unbound bile salts toward the canalicular and the biliary epithelium. Therapy with urso-deoxycholic acid (UDCA), which has favorable effects on the composition of bile, may be an effective treatment for patients with PFIC3. UDCA therapy has also been tried in children with PFIC1 or PFIC2, where its effectiveness seems to be related to the nature and severity of genetic abnormalities and the stage of disease at which UDCA is started.

An important question is whether heterozygous mutations of the genes that are responsible for the different types of PFIC could predispose to the development of cholestasis in other clinical settings. Homozygous mutations of the PFIC1 gene are considered to be responsible for the benign recurrent intrahepatic cholestasis in adults as discussed below. It is interesting that heterozygous mutations of the MDR3 gene have been detected in women with recurrent episodes of intrahepatic cholestasis of pregnancy. Thus, in the future, polymorphisms of the hepatobiliary transporters genes might be found to influence the individual susceptibility to several potentially cholestatic agents.

INBORN ERRORS IN BILE ACID SYNTHESIS

Inborn errors in bile acid synthesis represent familial conditions which are clinically characterized by progressive cholestatic disease. Specific defects have been identified in the enzymes that catalyze reactions in the pathway of formation of cholic and chenodeoxycholic acids. Cholestasis and liver injury are probably due to the absence or significant reduction of the primary bile acids or due to accumulation of potentially hepatotoxic intermediate bile acids. UDCA therapy results in normalization of biochemical abnormalities and improvement of histological lesions, particularly in case of early administration. Of interest is the observations that many chronic liver diseases may be associated with a secondary deficiency in 5β-reductase, which might be important for progression of liver damage.

CHRONIC CHOLESTATIC DISEASES IN ADULTHOOD

Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and drug-induced cholestasis are responsible for the majority of cases of chronic cholestatic disorders in adulthood, particulalry whenever extrahepatic obstruction of the bile ducts is excluded. The progress in our understanding and classification of these disorders are discussed below. Moreover, it should be briefly commented that the genetic basis for the relatively rare disorder of benign recurrent intrahepatic cholestasis (BRIC) has been recently found to be mutations in the PFIC1 gene. BRIC is characterized by episodes of cholestasis starting during adolescence or early adulthood and intermittent asymptomatic periods lasting for several months or years, but, in contrast to PFIC1, it is not associated with progressive liver disease.

PRIMARY BILIARY CIRRHOSIS

PBC is a chronic cholestatic liver disease caused by immune-mediated granulomatous destruction of intrahepatic bile ducts. The natural history of PBC is extremely variable and usually long, but its course is generally considered to be slowly progressive with development of fibrosis, cirrhosis, and eventually liver failure. A diagnostic feature of PBC is the detection in serum of anti-mitochondrial antibodies (AMA). We know now that these antibodies are mainly directed against the E2 components of the pyruvate dehydrogenase complex (anti-M2)

and/or the E3 binding protein (protein X). Anti-M2 represent the most specific serological marker for PBC and may be detected using several techniques (Elisa, Western-Blot, immunofluoresence). Other autoantibodies have also been identified in PBC patients (against several nuclear proteins such as gp120, p62, Sp100, PML), but they are not helpful in clinical practice. Although the presence of AMA or more specifically of anti-M2 indicates a specific immune response to mitochondrial antigens, there has been no firm evidence to support the involvement of these antibodies in the destruction of the intrahepatic bile ducts. Thus, although many data suggest that PBC is associated with inherited abnormalities of immunoregulation, its pathogenesis remains mostly unclarified.

About 10% of PBC patients are negative for AMA; anti-M2 may be detected in some, but not all, of such AMA-negative PBC patients. During the last decade, the term **autoimmune cholangitis** or **autoimmune cholangiopathy** has been used to describe these AMA-negative PBC patients.[7] Anti-nuclear (ANA) or anti-smooth muscle (SMA) antibodies are usually detected in this setting, but the clinical presentation and course of the liver disease are similar to those of PBC and not to those of autoimmune hepatitis. However, overlap syndromes that present with features of both PBC and autoimmune hepatitis may also develop and the classification of an individual patient may be difficult. It has been recently suggested that patients with the diagnosis of **PBC-autoimmune hepatitis overlap syndrome** must have at least two of the three diagnostic criteria for each condition (PBC: serum alkaline phosphatase levels >2xupper limit of normal, positive AMA, florid bile duct lesions in the liver biopsy; autoimmune hepatitis: alanine aminotransferase >5xupper limit of normal, positive ANA or SMA or serum immunoglobulin G levels >2xupper limit of normal, liver histology compatible with autoimmune hepatitis). It should be noted that the features of PBC and/or autoimmune hepatitis may present simultaneously or consecutively. The early recognition of these syndromes has not only theoretical interest, but therapeutic implications as well, since patients with PBC or autoimmune cholangitis should usually be treated only with UDCA and patients with PBC-autoimmune hepatitis overlap syndrome require a combination of prednisolone (or other immunosuppressive drug) and UDCA[8].

UDCA is currently the only approved pharmacological treatment for PBC and it is recommended for all patients with PBC, except those in the terminal phase of disease who require liver transplantation. UDCA has been suggested to have anticholestatic effects due to cytoprotective mechanisms on cell membranes, and stimulation of impaired hepatocellular secretion of hydrophobic bile acids and other potentially toxic cholephils; it may also have immunomodulatory effects. UDCA treatment has been consistently shown to improve the biochemical liver function tests of patients with PBC, but its effect on patient survival remains uncertain. A beneficial effect of UDCA therapy on transplantation free survival has been mostly supported by the combined analysis of three randomised trials,[9] but no evidence of therapeutic benefit was found in a recent meta-analysis[10]. In the longest study reported so far in PBC, we recently found that UDCA therapy does not have a demonstrable effect on the development of liver decompensation and on patient survival.[11] However, whether UDCA should not be offered to patients with PBC because of its cost, or whether it should be used even as placebo, given its safety and excellent tolerance cannot be easily answered.

PRIMARY SCLEROSING CHOLANGITIS

PSC is a chronic process of unknown origin characterized by segmental fibrosing inflammation of extrahepatic and/or intrahepatic bile ducts. Although PSC may occur alone, it is more commonly found in association with inflammatory bowel disease (IBD). It is estimated that the prevalence of IBD in PSC patients is as high as 55-75%. Recently, de novo IBD has been reported in patients transplanted for PSC. Thus, it appears that an even closer relationship between PSC and UC might exist if patients with PSC live long enough to express the colonic disease. On the other hand, although PSC is the most common clinically important liver disease and possibly the most common extraintestinal complication of IBD, it occurs in a minority of patients with IBD. PSC is found in 2.5-7.5% of ulcerative colitis (UC) and in 1% of Crohn's disease (CD) patients. In particular in patients with CD, PSC has been associated almost exclusively with disease of the colon.

Currently the etiopathogenesis of PSC is unknown, but the fact that the majority of PSC patients have IBD suggests similar factors may be involved in the pathogenesis of both diseases[12]. There is a genetic predisposition to develop PSC and IBD and both diseases are associated with immunologic disturbances. An increase in the prevalence of PSC and IBD in the relatives of index cases has been reported in many studies. Furthermore, occurrence of PSC and UC in members of the same fam-

ilies has also been observed. In recent years, although a possible role of portal infectious agents or toxins from increased intestinal permeability has fallen out of favor, there is still evidence to suggest similar pathogenetic factors between PSC and UC. There is an increased frequency of HLA haplotypes B8, DR3, DRW-52A and DR2 in PSC and of DR2 in UC. Similar pictures of both diseases can be produced by ischaemic insults and by infections, particularly in immunocompromised individuals. In addition, there are many features seen in both diseases that suggest autoimmunity may play an important role in the pathogenesis of both UC and PSC. A number of studies have speculated that cross-reactive pathogenic autoimmunity may be responsible for PSC in UC patients. Circulating autoantibodies have been described in both PSC and UC. Perinuclear antineutrophilic cytoplasmic antibodies (pANCA) are present in up to 85% of patients with both UC and PSC and anticolon antibodies reactive against protein P40, a putative colonic autoantigen, also react against biliary epithelium. The observations that IBD may develop after liver transplantation for PSC and that PSC may present after proctocolectomy for UC further favor the hypothesis that there is a common pathogenetic mechanism for both diseases. If autoimmune mechanisms are involved, there may be similar target antigens in the different organs affected. Autoimmunity does not seem to be important in CD and thus no clear explanation for a possible relationship between CD and PSC has been proposed.

PSC usually has an episodic course with recurrent attacks of cholangitis until biliary cirrhosis develops. Typically, the disease first presents in young men with symptoms of cholestasis in the majority of cases, but the diagnosis may be made in completely asymptomatic individuals as well, particularly in patients with UC and increased serum alkaline phosphatase levels. The diagnosis is based on cholangiographic (localized or multifocal strictures of extra- and/or intra-hepatic bile ducts with intervening segments of normal, diverticulum-like outpouchings, or ectatic ducts) and histological (fibrooblitterative ductal lesions) findings. It should be noted that the liver histology may be normal in about 5-10% of cases mainly due to the focal nature of the disease, and, conversely, cholangiography may reveal no abnormality in about 10-15%. In the latter cases, the diagnosis is based on the compatible clinical and biochemical profile of the patient and mainly on the liver biopsy; the entity is called small-duct PSC (previously known as pericholangitis)[13] and may represent an earlier phase of the disease, at least in some patients. Since non-organ specific autoantibodies (such as ANA or SMA) are often detected in PSC patients, small-duct PSC may be easily misdiagnosed as autoimmune hepatitis, at least in the beginning of its course. However, as in PBC, overlap syndromes that fulfil criteria for both PSC and autoimmune hepatitis have also been described.[14]

Currently, there is no treatment that may alter the course of PSC. Liver transplantation is the only therapeutic option that extends survival in PSC patients with end-stage liver disease. A complication specific to PSC is the high risk of development of cholangiocarcinoma, which is believed to develop in about 10-15% of PSC patients. Since the early diagnosis of cholangiocarcinoma is almost impossible, early consideration for liver transplantation could be an option for these patients. However, there are several points for which early transplantation for PSC because of the risk of cholangiocarcinoma remains a quite controversial issue. Apart form the increased risk of cholangiocarcinoma, the risk of colorectal cancer may also be higher in patients with IBD and PSC than with IBD alone and an even higher risk of colorectal cancer has been reported after liver transplantation in this setting, particularly within the first 2 years after transplant.[12,15] Although patients with PSC usually have mild or asymptomatic but extensive colitis, IBD often has an aggressive course after liver transplantation despite the administration of immunosuppressive drugs.[15] Recurrence of PSC after transplant has also been reported in up to 20% of patients, but, fortunately, it has not been associated with a significant decrease in the overall patient and graft survival.

DRUG-INDUCED CHOLESTASIS

Drugs may cause any type of acute or chronic liver disease. Acute cholestatic liver damage presents with pruritus and jaundice resembling an acute extrahepatic cholestatic disorder. The course of acute drug-induced cholestatic liver disease is usually self-limited with good prognosis and overall mortality in cases with jaundice of about 1%. However, the course of acute disease may be quite protracted and chronic cholestatic disorders may develop in a minority of cases despite discontinuation of the responsible drug. Chronic drug-induced cholestatic disorders can be classified into lesions of chronic intrahepatic cholestasis (with or without hepatocellular inflammation) and into lesions of extrahepatic cholestasis or PSC like lesions. Cases of intrahepatic cholestasis have a better prognosis, since improvement usually follows slowly the discontinuation of the responsible drug, except for cases with development of ductopenia. Ductopenic cholestasis and its main representative the vanish-

ing bile duct syndrome presents with clinical and biochemical features of cholestasis that persist for months or years after drug withdrawal and may lead to the development of biliary cirrhosis.

Development of chronic cholestatic disorders have been reported in association with treatment of several drugs, such as chloropromazine, amitriptyline, imipramine, carbamazepine, phenytoin, aloperidin, amoxycillin-clavulanic acid, erythromycin, ampicillin, tetracycline, trimethoprim-sulphamethoxazole, anabolic steroids, contraceptives, flucloxacillin, cyproeptadine, tolbutamide, thiobendazole. The exact pathogenetic mechanisms by which drugs cause cholestasis have not been identified for most of the cases. In a few cases, however, drug-induced inhibition of specific transporters has been identified. Thus, it seems that cyclosporin, rifampicin and rifamycin cause cholestasis by inhibition of the canalicular bile salt transport, mainly due to inhibition of BSEP[2].

Besides drug-induced cholestasis, the role of hepatobiliary transporters has been an attractive research field in several other cholestatic conditions in adulthood. Endotoxin-induced cholestasis has been shown to be associated with impairment of both bile-salt dependent and bile-salt independent bile formation mainly via reduction of NTCP, MRP2 and BSEP activity. Moreover, estrogen-induced cholestasis, such as intrahepatic cholestasis of pregnancy, has been associated with defective BSEP function[2]. Thus, one could speculate that heterozygous mutations or genetic polymorphism of the hepatobiliary transporters genes might be responsible for the individual sensitivity for development of cholestasis following stimulation of several potentially cholestatic agents.

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