Published in final edited form as:
Curr Treat Options Gastroenterol. 2007 December ; 10(6): 495–503.

The multiple facets of ABCB4 (MDR3) deficiency

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Opinion Statement

ABCB4 (MDR3) is a lipid translocator that moves phosphatidylcholine from the inner to outer leaflet of the canalicular membrane for extraction into the lumen by bile salts. Genetic mutations of ABCB4 lead to 3 distinct but related hepatobiliary diseases. Progressive familial intrahepatic cholestasis (PFIC) type 3 is a chronic cholestatic syndrome characterized by a markedly elevated GGTP. Patients present later in life than other types of PFIC with jaundice, pruritis and hepatosplenomegaly. Peri-portal inflammation progresses to biliary cirrhosis and eventually causes portal hypertension. Ursodeoxycholic acid (UDCA), which alters biliary bile composition, normalizes liver function tests in approximately half of treated PFIC type 3 patients. Partial or non-responders will eventually require liver transplantation. Gallstone patients with ABCB4 mutations may have Low Phospholipid Associated Cholelithiasis Syndrome. This is characterized by cholesterol gallstones and intrahepatic microlithiasis, along with recurrent biliary symptoms, despite cholecystectomy. A distinct group of patients with ABCB4 mutations may also develop intrahepatic brown pigment stones. The current treatment for these two diseases is UDCA, which may improve biliary symptoms even before the dissolution of stones occurs. Additional therapies such as Farnesoid X receptor ligands/agonists and benzfibrates show promise as future therapeutic modalities. Intrahepatic cholestasis of pregnancy affects pregnant women with abnormal ABCB4 variants. These women suffer from disabling pruritis and may experience steatorrhea as well. Their fetuses are at high risk for prematurity and still births. The definitive treatment for this condition is delivery of the baby. In the interim, limited fat intake, fat soluble vitamin supplementation and UDCA, with or without S-adenosylmethionine can provide symptomatic relief. In the future, additional hepatobiliary diseases related to ABCB4 mutations are likely to be identified. It is likely that this will result in the discovery of additional therapies for PFIC Type 3, gallstones and intrahepatic cholestasis of pregnancy.

Introduction

Defects in ATP binding cassette (ABC) transporters have been linked to several inherited cholestatic disorders. ABCB4 is exclusively expressed in the cannalicular membrane of the hepatocyte [1, 2]. It is a lipid translocator (or flippase) that moves the phospholipid phosphatidylcholine (PC) from the inner leaflet to the outer leaflet of the membrane, where it is then available for extraction by bile salts into the canalicular lumen. The exact mechanism by which bile salts extract PC is not clearly understood. In the canalicular lumen, PC is incorporated into mixed micelles, thereby reducing bile salt detergent activity, which could result in toxicity to bile duct epithelial cells [3].

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The nomenclature for ABCB4 has undergone multiple changes while its function was being elucidated. This lipid translocator was originally studied in rodent models and called Multi-Drug Resistance 2 (MDR-2) P glycoprotein, with the analogous human counterpart named MDR-3 P-glycoprotein. Current nomenclature now designates this PC translocator abcb4 in animals and ABCB4 in humans. The ABCB4 gene has been localized to chromosome 7q21.

Physiology and Regulation

An understanding of the function ABCB4 has come from studies conducted in abcb4 knockout mice. Abcb4 knockout mice develop liver disease very early in life, with evidence of hepatocyte necrosis, dilated canaliculi, significant portal tract inflammation and severe bile ductular proliferation. It has been speculated that the lack of abcb4 leads to hepatic accumulation of potential carcinogens. By 4-6 months of age, these mice form nodules that later develop hepatocellular carcinoma [4]. In abcb4 knockout mice, PC is not excreted normally into bile, whereas wild type mice on the same genetic background excrete PC into bile normally [5, 67]. The inability to excrete PC into bile can be overcome by the transgenic over expression of human ABCB4 [1, 5].

ABCB4 is a specific translocase for phosphatidylcholine. Transfecting polarized cells with ABCB4 increases the rate of transport of fluorescent labeled PC, but not other phospholipids, such as phosphatidylethanolamine [2, 8, 9]. The histologic pattern of PFIC type 3 is very similar to the MDR 2 knockout mouse [7]. Interestingly, abcb4 knockout mice also demonstrate decreased cholesterol excretion, proof that PC in mixed micelles is necessary to allow adequate biliary excretion of cholesterol. Bile salt micelles have a limited ability to solubilize cholesterol without the presence of PC [10]. PC and cholesterol excretion are, however, independent. This has been shown by infusion of hydrophobic bile salts like taurodeoxycholate into abcb4 knockout mice, which allows them to excrete cholesterol [10].

The expression of abcb4 can be induced by bile salts in rodents through the regulation of nuclear hormone receptor Farnesoid X Receptor (FXR) [1112]. Induction of abcb4 occurs with cholate feedings, but not ursodeoxycholic acid feeding. Cholate is a weak ligand and chenodeoxycholate is a strong ligand for FXR, while ursodeoxycholic acid is not an FXR ligand [8]. The human ABCB4 gene also has a functional FXR response element [13].

Mediated by PPAR-alpha (peroxisome proliferator activated receptor alpha), fibrates can also induce abcb4 in mice [14, 15]. Human PPAR-alpha activation patterns differ from mice, so that benzfibrates do not induce ABCB4 expression in man [16]. In the human HepG2 cell line (hepatoblastoma cells), benzfibrates induce redistribution of ABCB4 into pseudocanaliculi, which is attenuated by suppression of PPAR-alpha expression [17].

Genetic mutations of ABCB4 leads to 3 distinct but related hepatobiliary diseases: progressive familial intrahepatic cholestasis type 3 (PFIC Type 3), gallstones and intrahepatic cholestasis of pregnancy.

PFIC Type 3

Clinical Presentation

- PFIC Type 3 is an autosomal recessive chronic cholestatic syndrome, with a 25 % recurrence rate in affected families.
- PFIC Type 3 is characterized by a markedly elevated serum GGTP, in contrast to the low GGTP seen in PFIC Types 1 and 2. Patients present with a constellation of symptoms including jaundice, a mild and variable pruritis, discolored stools, hepatomegaly and splenomegaly.

- In contrast to other types of PFIC, children with PFIC Type 3 on average present symptomatically at a later age (mean of 39 months, range: 1 month to 20.5 years) [18]. However, patients with truncated proteins secondary to homozygous non-sense mutations manifest symptoms at a mean of 8 months of age, while patients with homozygous or heterozygote missense mutations present with symptoms later, mean at 3.5 years.
- PFIC type 3 patients develop progressive peri-portal inflammation and biliary cirrhosis leading to portal hypertension. Compared to PFIC types 1 and 2, the progression of disease is slower, with liver failure developing at a later age in PFIC Types 3 [4, 18].

Laboratory Evaluation

- Laboratory evaluation typically shows an increased mean ALT (5× the upper limit of normal [ULN]), conjugated bilirubin (2× ULN), alkaline phosphatase (2× ULN) and GGTP (13× ULN), but with normal cholesterol levels [18, 19]. These patients have increased serum concentrations of primary bile acids, but normal concentrations of biliary bile acids.
- They have low or absent ABCB4 expression on liver biopsy and low biliary concentrations of phospholipids [19].
- Liver histology shows portal fibrosis, significant ductular proliferation and a mixed inflammatory infiltrate. In more advanced disease, significant portal fibrosis and biliary cirrhosis are seen [4, 18, 19].

Treatment

- Ursodeoxycholic acid (UDCA) is the primary treatment for PFIC Type 3 and may improve both clinical symptoms and liver function tests [20]. In PFIC 3, UDCA may alter biliary bile acid composition in favor of hydrophilic bile acids, which are less toxic to the bile duct epithelium [7, 21]. Jacquemin et al. found that 46% of patients with PFIC type 3 responded completely to UDCA (20-30 mg/kg/day divided BID), with normalization of liver function tests. An additional 31% had a partial response to therapy with an improvement but not normalization of their liver function tests.
- Non-responders may have complete defects of ABCB4 (non-sense mutation) and lack any PC secretion into bile. Supplementation with UDCA, therefore, may not be enough to reduce the toxicity of the bile salts in all patients.
- Responders to UDCA likely have missense mutations, with a partial defect allowing for some residual phospholipids secretion into bile [22, 23]. Supplementation with UDCA may be enough to decrease bile toxicity beneath a critical threshold level in this subset of patients [23].
- Partial biliary diversion, a proven therapy for PFIC types 1 and 2, is not recommended for PFIC type 3.
- Liver transplantation provides the best therapeutic option for patients who do not respond to UDCA or progress despite initial response. In addition to cadaveric liver transplant, living related donor transplant should be considered for PFIC type 3 patients. The heterozygote ABCB4 state in donors has no adverse effect on the outcome of transplanted patients [24].
- Hepatocyte transplantation/regeneration in abcb4 knockout mice also shows promise [25]. Although hepatocyte transplantation for this particular disease has not been studied in humans, it may provide a therapeutic option in the future.

- Jung et al recently reported using prenatal mutation analysis in already affected kinships to identify fetuses with PFIC, including type 3. As the morbidity and mortality of PFIC type 3 is high, prenatal testing may allow families to make decisions about completion of affected pregnancies [26].

Gallstones

Pathophysiology

- Ethnic and familial clustering of gallstones led to the hypothesis that genetic susceptibility may predispose certain patients to gallstone formation. As discussed above, abcb4 knockout mice are unable to secrete PC into bile. These mice develop spontaneous gallstones after just 15 weeks on a chow diet. [27]. Biliary cholesterol solubilization depends on not only the concentration of cholesterol, but also bile salt and phospholipids concentrations. These substances form mixed micelles, and allow for maximal cholesterol solubilization. Decreased phospholipid concentrations in bile cause micellar destabilization and increased bile lithogenicity, therefore promoting cholesterol crystallization [4].
- In patients with ABCB4 defects, decreased biliary phospholipids, with relatively high cholesterol concentrations (i.e., a high cholesterol to PC ratio) results in a high cholesterol saturation index, promoting cholesterol crystallization and stone formation [8].

Clinical Presentation

- Several investigators have studied the genetic basis of gallstones. Rosmorduc et al. found ABCB4 mutations in a conserved amino acid region in a small group of patients with cholesterol gallstones and low concentrations of biliary phospholipids. Each patient had symptom onset prior to age 40, cholesterol gallstones and intrahepatic sludge/microlithiasis (as seen by hyperechoic foci on radiologic exams) and recurrent biliary symptoms even after cholecystectomy. The term “LPAC (Low Phospholipid Associated Cholelithiasis) Syndrome” was derived from this study [28]. Post-cholecystectomy biliary symptoms may be due to intrahepatic cholesterol deposits and bile duct inflammation, rather than detectable stones [29]. Homozygous and heterozygous mutations of ABCB4 were found in 56% of LPAC syndrome patients. No mutations were detected in asymptomatic patients or those with classic gallstones [29].
- Further support for the genetic basis of gallstones is implied by the occurrence of gallstones or intrahepatic cholestasis of pregnancy in individuals with a family history of PFIC type 3 [18, 30].
- A single patient has also been described who initially developed cholesterol cholelithiasis, followed by 2 episodes of intrahepatic cholestasis of pregnancy and then biliary cirrhosis later in life. This patient was confirmed by mutational analysis to have a heterozygote missense mutation of ABCB4 [31].
- Patients with intrahepatic brown pigment stones, distinct from the typical cholesterol stones, have also been found to harbor mutations in ABCB4 [32, 33]. These stones, prevalent in Japan, but uncommon in western countries, contain more cholesterol and less calcium bilirubinate and bile acids than the brown pigment stones that develop in extra hepatic bile ducts. Patients with these intrahepatic stones have decreased ABCB4 mRNA expression and PC transfer protein, reduced immunohistochemical staining for ABCB4 in liver tissue and decreased phospholipid content in bile [33]. Low phospholipid concentrations are detected in bile from hepatic ducts that contain stones as well as ducts that are unaffected [34].

These patients may experience long standing and often intractable cholangitis related to repeated intrahepatic lithiasis [34].

Treatment

- In LPAC syndrome, ursodeoxycholic acid (UDCA) may improve biliary symptoms even before intrahepatic stones resolve [28, 35].
- In animal models, abcb4 activity and expression can be induced by a variety of drugs. These include ligands for the bile salt receptor FXR, such as 6 alpha-hydroxylated bile salts and 6-alpha alkyl-substituted bile salts and synthetic agonists like GW4064. Cholesterol biosynthesis inhibitors like HMG- CoA reductase inhibitors, sqalene epoxidase inhibitors (Tu 2208) and oxidosqualene lanosterol cyclase inhibitors (Ro 48-8071) may also be beneficial [36].
- Fibrates are anti-hyperlipidemic agents that up regulate ABCB4 gene transcription via PPAR-alpha, with the potential to increase biliary phospholipids secretion [9, 14, 17]. Data using benzfibrates in primary biliary cirrhosis suggests that the function of ABCB4 can be augmented to increase PC secretion [37]. This therapeutic approach has not yet been formally studied in ABCB4 related stone disease, but holds promise.

Intrahepatic Cholestasis of Pregnancy

- Intrahepatic cholestasis of pregnancy (ICP) is a rare condition, seen in les than 1% of pregnancies in Europe and North America. In striking contrast, certain areas of Chile and Bolivia have rates as high as 16% [38].
- Approximately 30% of ICP cases have high GGTP levels, similar to patients with PFIC Type 3. Jacquemin et al. first implicated ABCB4 in the pathogenesis of ICP, reporting the coexistence of cases of PFIC type 3 and ICP in a large consanguineous family, which included individuals with a large frame shift mutation in ABCB4 [39]. Women who were heterozygous for this mutation developed ICP.
- Several other groups have reported additional mutations in ABCB4 associated with ICP [31, 40-43].
- Particular variants of ABCB4 may predispose women to particularly severe ICP [40].
- The heterozygote state for ABCB4 may represent a genetic predisposition which under the stress of pregnancy provides inadequate ABCB4 activity. Thus, women who have adequate ABCB4 under normal conditions, become symptomatic with addition of endogenous and exogenous alterations that occur during pregnancy [4, 9].

Pathogenesis

- Environmental factors are one such trigger that has been implicated in the pathophysiology of ICP. This is inferred by declining rates of ICP in Chile over the past decade, as well seasonal variation, with a higher incidence in the winter than summer [30, 44, 45]. Seasonal variations in ICP may be due to seasonal differences in selenium and copper contents of diets.
- Hormonal factors, both estrogen and progesterone, may also have an effect on the symptomatic presentation of ICP. ICP develops late in gestation, when hormone levels are at their highest, and resolves after delivery as hormone levels return to baseline values. Moreover, ICP occurs more frequently in twin pregnancies, in which hormone levels are higher [30, 44]. Estrogens are known to induce cholestasis

in experimental models of cholestasis [46]. Estrogens decrease uptake of bile acids at the hepatocyte basolateral membrane and inhibit expression of the canalicular bile salt export pump [47]. Increased sulfated progesterone metabolite levels are evident in patients with ICP [46]. These sulfated progesterones may saturate appropriate hepatic transporters in genetically predisposed individuals [48].

Clinical Presentation

- The primary presentation of ICP is pruritis, typically occurring in the third trimester. It is often most severe at night, with palms and soles most affected [38].
- Symptoms typically resolve with delivery and always by 2 weeks after delivery. If they do not, an alternative diagnosis should be considered.
- Jaundice is present in less than 10% of affected patients and occurs concurrent with pruritis.
- Some patients experience steatorrhea, which may be sub-clinical or result in fat soluble vitamin deficiency and weight loss. Replacing long chain triglycerides with medium chain triglycerides in those experiencing weight loss, as well as decreasing fat intake may help symptoms.
- Pregnant women with ICP with onset more than 3 weeks prior to delivery may require vitamin K to prevent post-partum hemorrhage [49].

Laboratory Evaluation

- ICP is a clinical diagnosis, based on symptoms and laboratory values. Although there are no specific radiologic findings, these women are more likely to have gallstones.
- Raised serum bile acid levels are the most sensitive indicator of ICP [30, 48]. AST and ALT may be increased 2-10× ULN, although serum bilirubin elevations are rare [30, 38]. Serum alkaline phosphatase levels are not reliable indicators of cholestasis in pregnancy because they are increased in normal pregnancies, caused by increased production by the placenta and bone. A serum GGTP level is useful, however, and may be normal or increased. If the GGTP levels are high, the patient likely has an ABCB4 mutation as an underlying cause of the ICP.
- Liver biopsy is rarely indicated, unless diagnostic confusion occurs.

Fetal Outcomes

- ICP recurs in 60-70% of subsequent pregnancies. ICP is generally considered a benign disorder for the mother.
- The fetus experiences the most significant consequences of ICP, with high rates of prematurity (19-60% of pregnancies), fetal distress and still births. Stillbirths occur 1-2% of pregnancies affected by ICP, usually after 35 weeks gestational age [3850]. Fetal complications rates have been correlated with maternal serum bile acid levels, especially if they are >40 umol/l [51]. Unfortunately, studies have shown that heightened surveillance of pregnancies complicated by ICP (using methods such as stress testing) does not decrease this risk.
- It has been suggested that affected pregnancies should be delivered by 38 weeks, and 36 weeks if the effect of ICP is severe. One must, however, carefully weigh the balance between premature delivery and the heightened risk of fetal complications seen with ICP.

Treatment

- The definitive treatment of ICP is delivery of the baby, after which symptoms resolve within two weeks.
- Prior to delivery, several therapies have been used to reduce the disabling pruritis. UDCA, a hydrophobic bile acid, is postulated to work by stimulating hepatobiliary secretion, protecting cholangiocytes against hydrophobic bile acids that are cytotoxic and protecting hepatocytes against bile acid induced apoptosis [52]. UDCA improves pruritis and biochemical cholestasis, without adverse effects on either or the mother or fetus [53, 5455]. Additionally, UDCA treatment may allow for longer in utero gestation (by 4 weeks), although other complications of ICP for the fetus are not reduced [53, 54]. Typically UDCA is dosed at 12-15 mg/kg/day although a single study has suggested that much higher doses (1.5-2 grams per day) are more effective [5644].
- S-adenosylmethionine (SAM) is involved in phosphatidylcholine synthesis in the liver and is protective against hormone associated cholestasis in animal models. Clinical trials have yielded conflicting results. A few randomized control trials found that SAM was more effective than placebo in treating ICP [57, 58]. Another randomized control trial of SAM versus placebo, however, failed to show a therapeutic benefit. SAM may be less effective in treating moderate to severe ICP, but acceptable for mild ICP associated pruritis [59].
- UDCA is more effective than either placebo or SAM in reducing pruritis and improving biochemical parameters of ICP [53, 54, 59, 60]. A combination of UDCA and SAM may provide the greatest improvement in pruritis, as well as biochemical parameters [57]. Roncaglia et al., in a single study, found that UDCA and SAM were equivalent in treating ICP associated pruritis, but that UDCA improved biochemical parameters more than SAM [61]. Binder et al. also compared UDCA, SAM and a combination of UDCA and SAM. They found that UDCA alone or a combination of UDCA and SAM resulted in the greatest improvement of clinical symptoms and biochemical parameters [59]. Thus, UDCA is generally used as a single agent, with the benefits of a combination of UDCA and SAM still not proven conclusively.
- Dexamethasone has also been used for treating ICP associated pruritis [62]. A recent randomized controlled trial comparing Dexamethasone to UDCA and placebo, however, found that dexamethasone was not effective in improving pruritis or ALT, and less effective than UDCA in decreasing bilirubin or bile acids levels [51].
- Cholestyramine and cholestipol, both bile acid binding resins, interrupt enterohepatic circulation and increase fecal excretion of these potential pruritogens. These medications provide minimal relief of pruritis and certainly less than UDCA [63]. Additionally, these drugs have fallen out of favor because they interfere with the absorption of fat soluble vitamins and put patients at risk for post-partum hemorrhage [44].
- As in many other cholestatic conditions, both phenobarbital and rifampin have been used to treat ICP associated pruritis. However, in ICP these drugs had minimal effect on pruritis and no effect on liver tests [44].
- Anti-histamines can be used as adjunctive therapy, primarily because of their sedating effects.

Conclusion

- The ABCB4 molecule has now been associated with at least 3 disease variants. It is likely that additional hepatobiliary diseases related to abnormal ABCB4 will continue to be identified. In addition, ABCB4 is a likely modifier gene in a variety of hepatobiliary disorders.

References

Articles of particular interest have been highlighted as: *

1. Smith AJ, de Vree JM, Ottenhoff R, Oude Elferink RP, Schinkel AH, Borst P. Hepatocyte-specific expression of the human MDR3 P-glycoprotein gene restores the biliary phosphatidylcholine excretion absent in Mdr2 (-/-) mice. Hepatology. 1998; 28(2):530–6. [PubMed: 9696021]
2. Ruetz S, Gros P. Phosphatidylcholine translocase: a physiological role for the mdr2 gene. Cell. 1994; 77(7):1071–81. [PubMed: 7912658]
3. Elferink RP, Tytgat GN, Groen AK. Hepatic canalicular membrane 1: The role of mdr2 P-glycoprotein in hepatobiliary lipid transport. Faseb J. 1997; 11(1):19–28. [PubMed: 9034162]
4. Jacquemin E. Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: one gene for three diseases. Semin Liver Dis. 2001; 21(4):551–62. [PubMed: 11745043]
5. Oude Elferink RP, Ottenhoff R, van Wijland M, Smit JJ, Schinkel AH, Groen AK. Regulation of biliary lipid secretion by mdr2 P-glycoprotein in the mouse. J Clin Invest. 1995; 95(1):31–8. [PubMed: 7814632]
6. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiol Rev. 2003; 83(2):633–71. [PubMed: 12663868]
7. Smit JJ, Schinkel AH, Oude Elferink RP, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell. 1993; 75(3):451–62. [PubMed: 8106172]
8. Oude Elferink RP, Paulusma CC. Function and pathophysiological importance of ABCB4 (MDR3 P-glycoprotein). Pflugers Arch. 2007; 453(5):601–10. [PubMed: 16622704]
9. Trauner M, Fickert P, Wagner M. MDR3 (ABCB4) defects: a paradigm for the genetics of adult cholestatic syndromes. Semin Liver Dis. 2007; 27(1):77–98. [PubMed: 17295178]
10. Oude Elferink RP, Ottenhoff R, van Wijland M, Frijters CM, van Nieuwkerk C, Groen AK. Uncoupling of biliary phospholipid and cholesterol secretion in mice with reduced expression of mdr2 P-glycoprotein. J Lipid Res. 1996; 37(5):1065–75. [PubMed: 8725158]
11. Gupta S, Todd Stravitz R, Pandak WM, Muller M, Reno Vlahcevic Z, Hylemon PB. Regulation of multidrug resistance 2 P-glycoprotein expression by bile salts in rats and in primary cultures of rat hepatocytes. Hepatology. 2000; 32(2):341–7. [PubMed: 10915741]
12. Frijters CM, Ottenhoff R, van Wijland MJ, van Nieuwkerk CM, Groen AK, Oude Elferink RP. Regulation of mdr2 P-glycoprotein expression by bile salts. Biochem J. 1997; 321(Pt 2):389–95. [PubMed: 9020871]
13. Huang L, Zhao A, Lew JL, et al. Farnesoid X receptor activates transcription of the phospholipid pump MDR3. J Biol Chem. 2003; 278(51):51085–90. [PubMed: 14527955]
14. Chianale J, Vollrath V, Wielandt AM, et al. Fibrates induce mdr2 gene expression and biliary phospholipid secretion in the mouse. Biochem J. 1996; 314(Pt 3):781–6. [PubMed: 8615769]
15. Kok T, Bloks VW, Wolters H, et al. Peroxisome proliferator-activated receptor alpha (PPARalpha)-mediated regulation of multidrug resistance 2 (Mdr2) expression and function in mice. Biochem J. 2003; 369(Pt 3):539–47. [PubMed: 12381268]
16. Roglans N, Vazquez-Carrera M, Alegret M, et al. Fibrates modify the expression of key factors involved in bile-acid synthesis and biliary-lipid secretion in gallstone patients. European journal of clinical pharmacology. 2004; 59(12):855–61. [PubMed: 14685799]
17. Shoda J, Inada Y, Tsuji A, et al. Bezafibrate stimulates canalicular localization of NBD-labeled PC in HepG2 cells by PPARalpha-mediated redistribution of ABCB4. J Lipid Res. 2004; 45(10): 1813–25. [PubMed: 15258199]

*18. Jacquemin E, De Vree JM, Cresteil D, et al. The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. Gastroenterology. 2001; 120(6): 1448–58. This important publication reported that at least one third of PFIC Type 3 patients have proven defects of ABCB4. [PubMed: 11313315]

19. Deleuze JF, Jacquemin E, Dubuisson C, et al. Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. Hepatology. 1996; 23(4):904–8. [PubMed: 8666348]

20. Jacquemin E, Hermans D, Myara A, et al. Ursodeoxycholic acid therapy in pediatric patients with progressive familial intrahepatic cholestasis. Hepatology. 1997; 25(3):519–23. [PubMed: 9049190]

21. Van Nieuwkerk CM, Elferink RP, Groen AK, et al. Effects of Ursodeoxycholate and cholate feeding on liver disease in FVB mice with a disrupted mdr2 P-glycoprotein gene. Gastroenterology. 1996; 111(1):165–71. [PubMed: 8698195]

22. de Vree JM, Jacquemin E, Sturm E, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci U S A. 1998; 95(1):282–7. [PubMed: 9419367]

23. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis. 2000; 4(4):753–63. [PubMed: 11232355]

24. Bassas A, Chehab M, Hebby H, et al. Living related liver transplantation in 13 cases of progressive familial intrahepatic cholestasis. Transplant Proc. 2003; 35(8):3003–5. [PubMed: 14697961]

25. De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology. 2000; 119(6):1720–30. [PubMed: 11113093]

26. Jung C, Driancourt C, Baussan C, et al. Prenatal molecular diagnosis of inherited cholestatic diseases. J Pediatr Gastroenterol Nutr. 2007; 44(4):453–8. [PubMed: 17414143]

27. Lammert F, Wang DQ, Hillebrandt S, et al. Spontaneous cholecysto- and hepatolithiasis in Mdr2-/- mice: a model for low phospholipid-associated cholelithiasis. Hepatology. 2004; 39(1):117–28. [PubMed: 14752830]

28. Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology. 2001; 120(6):1459–67. [PubMed: 11313316]

29. Rosmorduc O, Hermelin B, Boelle PY, Parc R, Taboury J, Poupon R. ABCB4 gene mutation-associated cholelithiasis in adults. Gastroenterology. 2003; 125(2):452–9. [PubMed: 12891548]

30. Lammert F, Marschall HU, Glantz A, Matern S. Intrahepatic cholestasis of pregnancy: molecular pathogenesis, diagnosis and management. J Hepatol. 2000; 33(6):1012–21. [PubMed: 11131439]

31. Lucena JF, Herrero JI, Quiroga J, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. Gastroenterology. 2003; 124(4):1037–42. [PubMed: 12671900]

32. Kano M, Shoda J, Sumazaki R, Oda K, Nimura Y, Tanaka N. Mutations identified in the human multidrug resistance P-glycoprotein 3 (ABCB4) gene in patients with primary hepatolithiasis. Hepatol Res. 2004; 29(3):160–6. [PubMed: 15203080]

33. Shoda J, Oda K, Suzuki H, et al. Etiologic significance of defects in cholesterol, phospholipid, and bile acid metabolism in the liver of patients with intrahepatic calculi. Hepatology. 2001; 33(5): 1194–205. [PubMed: 11343249]

34. Shoda J, Inada Y, Osuga T. Molecular pathogenesis of hepatolithiasis--a type of low phospholipid-associated cholelithiasis. Front Biosci. 2006; 11:669–75. [PubMed: 16146760]

35. Tomida S, Abei M, Yamaguchi T, et al. Long-term ursodeoxycholic acid therapy is associated with reduced risk of biliary pain and acute cholecystitis in patients with gallbladder stones: a cohort analysis. Hepatology. 1999; 30(1):6–13. [PubMed: 10385632]

36. Lammert F, Matern S. The genetic background of cholesterol gallstone formation: an inventory of human lithogenic genes. Current drug targets. 2005; 5(2):163–70. [PubMed: 16089348]

37. Kurihara T, Niimi A, Maeda A, Shigemoto M, Yamashita K. Bezafibrate in the treatment of primary biliary cirrhosis: comparison with ursodeoxycholic acid. Am J Gastroenterol. 2000; 95(10):2990–2. [PubMed: 11051391]

38. Riely CA, Bacq Y. Intrahepatic cholestasis of pregnancy. Clin Liver Dis. 2004; 8(1):167–76. [PubMed: 15062199]
39. Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. Lancet. 1999; 353(9148):210–1. [PubMed: 9923886]
40. Wasmuth HE, Glantz A, Keppeler H, et al. Intrahepatic cholestasis of pregnancy: the severe form is associated with common variants of the hepatobiliary phospholipid transporter ABCB4 gene. Gut. 2007; 56(2):265–70. [PubMed: 16891356]
41. Floreani A, Carderi I, Paternoster D, et al. Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. Alimentary pharmacology & therapeutics. 2006; 23(11):1649–53. [PubMed: 16696816]
42. Pauli-Magnus C, Lang T, Meier Y, et al. Sequence analysis of bile salt export pump (ABCB11) and multidrug resistance p-glycoprotein 3 (ABCB4, MDR3) in patients with intrahepatic cholestasis of pregnancy. Pharmacogenetics. 2004; 14(2):91–102. [PubMed: 15077010]
*43. Schneider G, Paus TC, Kullak-Ublick GA, et al. Linkage between a new splicing site mutation in the MDR3 alias ABCB4 gene and intrahepatic cholestasis of pregnancy. Hepatology. 2007; 45(1):150–8. This important publication demonstrates that splicing mutations in the ABCB4 gene can cause both intrahepatic cholestasis as well as gallstone disease. [PubMed: 17187437]
44. Lammert F, Marschall HU, Matern S. Intrahepatic Cholestasis of Pregnancy. Curr Treat Options Gastroenterol. 2003; 6(2):123–32. [PubMed: 12628071]
45. Reyes H, Baez ME, Gonzalez MC, et al. Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile. J Hepatol. 2000; 32(4):542–9. [PubMed: 10782901]
46. Reyes H, Simon FR. Intrahepatic cholestasis of pregnancy: an estrogen-related disease. Semin Liver Dis. 1993; 13(3):289–301. [PubMed: 8235718]
47. Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. Gastroenterology. 2000; 118(2):422–30. [PubMed: 10648470]
48. Reyes H, Sjovall J. Bile acids and progesterone metabolites in intrahepatic cholestasis of pregnancy. Annals of medicine. 2000; 32(2):94–106. [PubMed: 10766400]
49. Reyes H, Radrigan ME, Gonzalez MC, et al. Steatorrhea in patients with intrahepatic cholestasis of pregnancy. Gastroenterology. 1987; 93(3):584–90. [PubMed: 3609667]
50. Rioseco AJ, Ivankovic MB, Manzur A, et al. Intrahepatic cholestasis of pregnancy: a retrospective case-control study of perinatal outcome. American journal of obstetrics and gynecology. 1994; 170(3):890–5. [PubMed: 8141222]
51. Glantz A, Marschall HU, Lammert F, Mattsson LA. Intrahepatic cholestasis of pregnancy: a randomized controlled trial comparing dexamethasone and ursodeoxycholic acid. Hepatology. 2005; 42(6):1399–405. [PubMed: 16317669]
52. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology. 2002; 36(3):525–31. [PubMed: 12198643]
53. Diaferia A, Nicastri PL, Tartagni M, Loizzi P, Iacovizzi C, Di Leo A. Ursodeoxycholic acid therapy in pregnant women with cholestasis. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 1996; 52(2):133–40. [PubMed: 8855091]
54. Palma J, Reyes H, Ribalta J, et al. Ursodeoxycholic acid in the treatment of cholestasis of pregnancy: a randomized, double-blind study controlled with placebo. J Hepatol. 1997; 27(6): 1022–8. [PubMed: 9453428]
55. Zapata R, Sandoval L, Palma J, et al. Ursodeoxycholic acid in the treatment of intrahepatic cholestasis of pregnancy. A 12-year experience Liver Int. 2005; 25(3):548–54.
56. Mazzella G, Rizzo N, Azzaroli F, et al. Ursodeoxycholic acid administration in patients with cholestasis of pregnancy: effects on primary bile acids in babies and mothers. Hepatology. 2001; 33(3):504–8. [PubMed: 11230728]
57. Nicastri PL, Diaferia A, Tartagni M, Loizzi P, Fanelli M. A randomised placebocontrolled trial of ursodeoxycholic acid and S-adenosylmethionine in the treatment of intrahepatic cholestasis of

pregnancy. British journal of obstetrics and gynaecology. 1998; 105(11):1205–7. [PubMed: 9853771]

58. Frezza M, Centini G, Cammareri G, Le Grazie C, Di Padova C. S-adenosylmethionine for the treatment of intrahepatic cholestasis of pregnancy. Results of a controlled clinical trial Hepato-gastroenterology. 1990; 37(2):122–5.

*59. Binder T, Salaj P, Zima T, Vitek L. Randomized prospective comparative study of ursodeoxycholic acid and S-adenosyl-L-methionine in the treatment of intrahepatic cholestasis of pregnancy. Journal of perinatal medicine. 2006; 34(5):383–91. This important publication demonstrates the effectiveness of ursodeoxycholic acid in treating intrahepatic cholestasis, and that S-adenosylmethionine may have a synergistic effect. [PubMed: 16965225]

60. Palma J, Reyes H, Ribalta J, et al. Effects of ursodeoxycholic acid in patients with intrahepatic cholestasis of pregnancy. Hepatology. 1992; 15(6):1043–7. [PubMed: 1592342]

61. Roncaglia N, Locatelli A, Arreghini A, et al. A randomised controlled trial of ursodeoxycholic acid and S-adenosyl-l-methionine in the treatment of gestational cholestasis. Bjog. 2004; 111(1):17–21. [PubMed: 14687046]

62. Hirvioja ML, Tuimala R, Vuori J. The treatment of intrahepatic cholestasis of pregnancy by dexamethasone. British journal of obstetrics and gynaecology. 1992; 99(2):109–11. [PubMed: 1554659]

63. Kondrackiene J, Beuers U, Kupcinskas L. Efficacy and safety of ursodeoxycholic acid versus cholestyramine in intrahepatic cholestasis of pregnancy. Gastroenterology. 2005; 129(3):894–901. [PubMed: 16143129]

Table 1

Disease variants associated with the ABCB4 molecule.

Disease	Phenotype	Treatment
Progressive Familial Intrahepatic Cholestasis (PFIC) Type 3	Progressive cholestasis with high GGTP. Late development of hepatic inflammation and biliary cirrhosis leading to portal hypertension. Increased AST/ALT and serum bile acids with normal cholesterol and biliary bile acids.	UDCA Liver Transplantation
Cholelithiasis	LPAC Syndrome: onset of cholesterol gallstones and intrahepatic microlithiasis and recurrent biliary symptoms despite cholecystectomy at age < 40 years. Intrahepatic brown pigment stones with difficult to treat cholangitis.	UDCA Future therapies may include FXR ligands and agonists and benzfibrates.
Intrahepatic Cholestasis of Pregnancy	Third trimester pruritis. Jaundice is rare. +/− Steatorrhea. Raised serum bile acids. Increased risk of fetal prematurity and stillbirths. Symptom resolution after delivery.	Delivery of baby. UDCA +/− UDCA with SAM

Abbreviations: UDCA, Ursodeoxycholic Acid; FXR, Farnesoid X Receptor; SAM, S-adenosylmethionine