Molecular basis of cholestatic diseases of surgical interest

Luis Alvarez, PhD,[a] **Paloma Jara, MD,**[b] **Loreto Hierro, MD,**[b]
Elena Sánchez-Sabaté, MD,[a] **Pilar Martínez-Fernández, PhD,**[a]
Manuel López-Santamaría, MD[c]

[a]*From the Research Unit, La Paz Children's University Hospital, Madrid, Spain;*
[b]*Pediatric Liver Service, La Paz Children's University Hospital, Madrid, Spain; and the*
[c]*Department of Pediatric Surgery, La Paz Children's University Hospital, Madrid, Spain.*

INDEX WORDS
Biliary atresia;
Alagille syndrome;
JAG1;
Progressive familial
intrahepatic
cholestasis

Cholestasis constitutes one of the most common and severe manifestations of acquired or inherited liver disease. When manifest in early infancy, it is often life-threatening and usually requires surgical management. In many cases, liver transplantation is the only effective therapy. Extensive knowledge about the molecular mechanisms underlying several pediatric cholestatic disorders has been gained in recent years from studies in both experimental models and clinical forms. In this review, we focus on recent contributions to the knowledge of molecular basis of main pediatric cholestatic disorders, such as biliary atresia, Alagille syndrome, and familial intrahepatic cholestasis. For some of them, putative targets of therapeutic interest, such as interferon-γ and Farnesoid X receptor, have been proposed.
© 2005 Elsevier Inc. All rights reserved.

Cholestasis results from the impaired secretion of bile from the liver to the intestine. As such, it represents a clinical and biochemical syndrome that is produced by a wide variety of disease processes that affect the liver. Individuals with cholestasis manifest jaundice, severe itching, malabsorption of fats and lipid-soluble vitamins, and, in many cases, progressive liver damage. These clinical manifestations are due to accumulation in blood and tissues of substances normally secreted in the bile, namely bilirubin, bile acids, and cholesterol, and to the absence of bile from the intestine.

There are many causes of cholestasis in early infancy ranging from normal physiologic jaundice to complete obliteration of biliary tree. Intrahepatic cholestasis may re-

sult from occlusion or paucity of intrahepatic bile ducts or from functional failure of the hepatocytes to secrete bile. Obstruction of extrahepatic bile duct is usually referred to as extrahepatic cholestasis. Progress in the molecular mechanisms underlying disorders that fall into these categories and often require liver transplantation forms the basis of this review.

Biliary atresia

Biliary atresia represents the most frequent cause of neonatal cholestasis. It is a devastating disease resulting from a fibroinflammatory obliteration of the bile duct system that leads to impaired bile flow and ongoing hepatocellular injury.[1] For affected children to survive, the only effective treatment is surgical. The bile duct obstruction may be relieved by portoenterostomy (the Kasai's procedure), which in most cases improves bile drainage, particularly if

Address reprint requests and correspondence: Luis Alvarez, PhD, Research Unit, La Paz University Hospital, Paseo de La Castellana, 261, 28046 Madrid, Spain.
E-mail address: luisalvarez.hulp@salud.madrid.org.

it is performed within the first months of life.[2] However, this is a temporary treatment, and the majority of patients develop progressive hepatic fibrosis and eventually require liver transplantation.[3] In fact, biliary atresia remains the most common indication for liver transplantation in childhood. Two clinical forms of biliary atresia are currently recognized: the fetal or embryonic form and the perinatal one.[1,3] In both cases, the cause of disease remains unknown. Although it typically presents soon after birth, it does not appear to be an inherited disease. The embryonic form (also termed "polyasplenia syndrome" or "biliary atresia splenic malformation syndrome") has been related to defects in embryogenesis, since it is frequently associated with other congenital anomalies, including poly- or asplenia, situs inversus, and cardiac abnormalities.[4] A putative role of genes regulating laterality was suggested from findings in mice carrying a mutation in the inversin (*Inv*) gene, which present abnormalities in organ symmetry and obliteration of extrahepatic bile ducts.[5] However, the involvement of *INV* in the embryonic form of biliary atresia seems unlikely, since no mutation in this gene has been found in affected children.[6] Heterozygous mutations in genes involved in establishing the left–right axis, such as *CFC1*, which encodes the CRYPTIC protein, have been detected in several individuals with biliary atresia and laterality defects.[7,8] But these mutations are suggested to confer a predisposition rather to produce the disease phenotype. To date, no consistent association between mutations in genes regulating laterality and the development of biliary atresia has been reported. Recently, Zhang and coworkers have suggested that phenotypic manifestations of the embryonic form of biliary atresia are modulated by epigenetic factors, since livers from affected children exhibit an increased expression of genes involved in chromatin function and imprinted genes, as compared with livers from patients with the perinatal form.[9] Such a possibility is highly suggestive, but has yet to be adequately investigated.

Children with the perinatal form of biliary atresia do not present other congenital anomalies. Different etiologies have been postulated for the disease, including vascular lesions, environmental toxins, aberrant immune and/or inflammatory response, and occult viral infections.[3,10] The current theories suggest that biliary atresia is not a single disease, but rather a heterogeneous condition resulting from a complex interplay among genetic factors, insults that target the hepatobiliary tree, and activation of particular immunologic pathways.[3,10,11]

Regardless of the initiating insult, there is increasing evidence pointing to the activation of a cell-mediated immune response as a determinant factor in the pathogenesis of biliary atresia. Earlier studies showed that bile duct damage in children with biliary atresia was associated with lymphocytic infiltration into bile duct epithelium.[12,13] The cell infiltrates were subsequently demonstrated to be predominantly Kupffer cells (resident liver macrophages), and CD4$^+$ (helper), CD8$^+$ (cytotoxic), and natural killer lymphocytes.[14,15] A functional commitment of lymphocytes has been supported by gene-profiling analyses performed in liver biopsies from infants with biliary atresia at early stages of disease and age-matched diseased control subjects. Samples from children with biliary atresia exhibited a coordinated overexpression of genes regulating lymphocyte differentiation, such as osteopontin, a regulator of cell-mediated immunity, and interferon-γ (IFN-γ).[16] Whether hepatic inflammation of children with biliary atresia represents a specific immune process involved in the pathogenesis of the disease or a secondary response to cholestasis has been recently addressed. Mack and coworkers have found that portal tracts of children with biliary atresia at the time of diagnosis (3-12 weeks of age) are infiltrated with a characteristic inflammatory cell population (CD4$^+$ and CD8$^+$ T cells, and Kupffer cells), with local production of proinflammatory cytokines, such as IL-2, IFN-γ, and tumor necrosis factor-α.[17] This pattern was not found in liver from patients with other neonatal cholestatic disorders (including extrahepatic bile duct obstruction by a choledocal cyst), therefore suggesting that portal tract inflammation in biliary atresia is not secondary to the presence of cholestasis, but rather it is an event involved in the pathogenesis of the disease. Further experimental evidence has been provided by Shivakumar and coworkers in a murine model of rotavirus-induced biliary atresia. Rotavirus infection of neonatal mice specifically targeted bile ducts cells and triggered an immediate infiltration of the hepatobiliary system by neutrophils, followed by IFN-γ-producing T lymphocytes, which resulted in obstruction of extrahepatic bile ducts. IFN-γ was demonstrated to play a key regulatory role in the pathogenesis of bile duct injury and obstruction, since loss of expression of this cytokine prevented the inflammatory and fibrosing occlusion of bile ducts.[18] Although, as noted above, an increased expression of IFN-γ has been found in infants at early stages of disease,[16] it is difficult to assess whether IFN-γ also regulates biliary obstruction in humans. Nevertheless, altogether these findings offer the encouraging prospect that selective blockage of IFN-γ action may be an approach to management of this disease.

Alagille syndrome

Alagille syndrome is an autosomal dominant disorder characterized by developmental abnormalities of the liver, heart, face, eye, kidney, and skeleton. This disease exhibits extremely variable expressivity ranging from apparent normal phenotype to severely affected cases; some patients present only congenital cardiovascular defects such as tetralogy of Fallot and pulmonary artery hypoplasia or stenosis.[19] The hepatic manifestations result from a paucity of intrahepatic bile ducts and vary from mild to severe cholestasis.[20] Liver transplantation is eventually necessary in 30% to 50% of patients who have hepatic symptoms in infancy.[21,22] Alagille syndrome is caused by mutation or deletion of one copy of *JAG1* (*Jagged1*) gene, which probably leads to
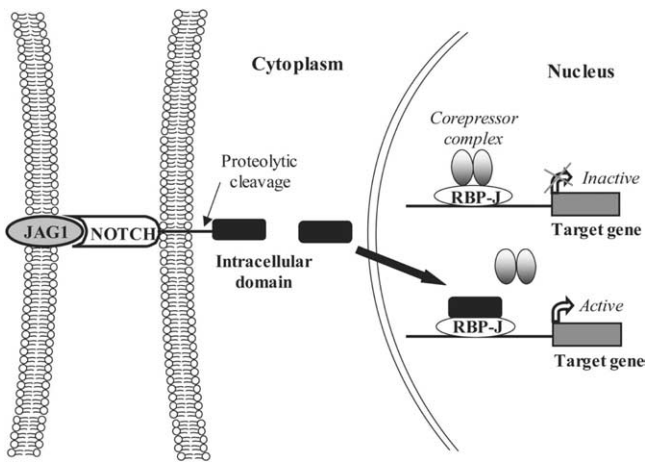
Figure 1 Simplified overview of the Notch signaling pathway in mammals. Interaction of JAG1 with Notch receptors expressed on adjacent cells leads to transcriptional activation of target genes involved in cell differentiation. See the text for details.

protein haploinsufficiency.[23,24] *JAG1* encodes a cell surface protein that functions as a ligand for the Notch transmembrane receptors. Interaction of Notch receptors (Notch 1 to 4) with their ligands (JAG1, JAG2, Delta-like1, Delta-like3, Delta-like4) represents an evolutionary conserved cell-to-cell communication system that plays a critical role in cell fate determination and differentiation.[25]

In the liver, JAG1/Notch signaling pathway appears to be involved in bile duct morphogenesis and/or the maintenance of the differentiated phenotype of biliary epithelium.[26] A putative role in the postnatal development of intrahepatic bile ducts is suggested by findings showing that bile ducts are not congenitally lacking in patients with Alagille syndrome, but that the ductal paucity develops progressively after birth.[19] Consistent with this, it has been recently reported that Notch signaling pathway is activated in mice during neonatal period by interaction between Jag1 expressed in the periportal mesenchyme and Notch2 receptor located in the adjacent biliary epithelial cells.[27] However, expression patterns of JAG1 and Notch receptors in the developing human liver have raised the possibility that JAG1/Notch signaling also influences duct formation during embryogenesis. Thus, in the human fetal liver, at the time of ductal plate formation, Notch3 receptor has been detected in mesenchymal cells located in the vicinity of ductal plate cells that expresses JAG1.[28] Further studies are needed to fully define the temporal and spatial specific interactions between JAG1 and Notch receptors and the stage(s) at which Notch signaling pathway is involved in biliary development. Nevertheless, it is clear that the molecular outcome of JAG1–Notch interaction is the activation of key genes involved in cell differentiation. The chain of intracellular events triggered on binding of JAG1 to Notch receptors expressed on adjacent cells is illustrated in Figure 1. In the absence of Notch signaling, transcription of primary target genes is silenced by a corepressor complex, which includes hystone deacetylases, recruited by the transcription

factor RBP-J (also known as CSL). The receptor/ligand interaction induces the proteolytic cleavage of the receptor that releases its intracellular domain. This domain migrates into the nucleus and displaces the corepressor complex from the RBP-J protein, leading to transcriptional activation of the target genes.[25,29,30]

Although the implication of *JAG1* in Alagille syndrome has been widely evidenced and tissue distribution of JAG1 is consistent with clinical abnormalities observed in affected patients,[31,32] there is no clear genotype–phenotype correlation and, as stated earlier, individuals carrying identical mutations may have highly variable manifestations. This has been best exemplified in a case of monozygotic twins with discordant Alagille phenotypes: one twin presented severe pulmonary atresia and mild liver disease; the other twin had tetralogy of Fallot and severe cholestasis that required liver transplantation.[33] Therefore, identification of a particular *JAG1* mutation does not seem to offer any prediction of the severity of the disease. The variable phenotypes could most likely be explained by the existence of either genetic or environmental modifiers, or the interaction of both. This is supported by studies with mouse models. Mice heterozygous for *Jag1* mutation, whose genotype mimics that of patients with Alagille syndrome, exhibit only ocular defects, and do not manifest other phenotypes associated with Alagille syndrome in humans.[34] However, mice doubly heterozygous for a *Jag1* null allele and a *Notch2* hypomorphic allele exhibit the developmental abnormalities characteristic of Alagille syndrome.[35] Thus, *Notch2* gene appears to act as a genetic modifier to interact with a *Jag1* mutation in mice. This raises the possibility that polymorphisms in particular *Notch2* alleles, or in genes encoding other components of the Notch signaling pathway, may influence the severity of Alagille syndrome.

Progressive familial intrahepatic cholestasis

Progressive familial intrahepatic cholestasis (PFIC) is a clinical term that encompasses a subset of hereditary disorders caused by impaired bile flow without anatomic obstruction.[36] Affected children typically present chronic intrahepatic cholestasis early in infancy, which leads to end-stage liver disease. Jaundice, severe pruritus, and failure to thrive are the common and predominant symptoms.[37] This heterogeneous group is currently separated into different genetic diseases, inherited in a autosomal-recessive fashion. They include BSEP disease and FIC disease, which are characterized by low serum concentrations of γ-glutamyl transpeptidase activity and cholesterol despite conjugated hyperbilirubinemia and by decreased concentrations of bile salts in bile.[38,39] BSEP disease is caused by mutation in *ABCB11* gene, which encodes the primary bile salt export pump (BSEP). It is localized in the canalicular membrane of hepatocytes and functions in transporting bile acids out of the hepatocyte into the canaliculus.[40,41] Hepatocellular retention of bile
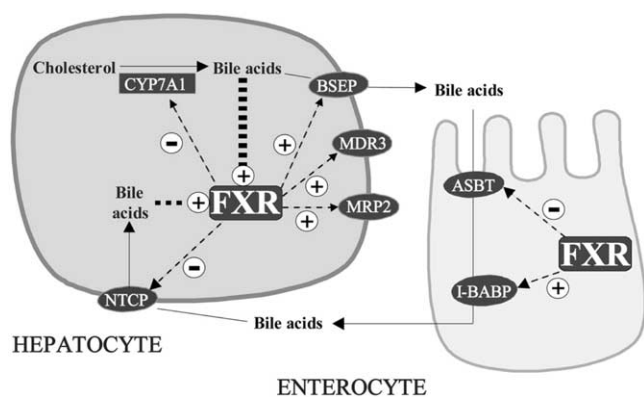
Figure 2 FXR coordinately regulates bile acid metabolism and enterohepatic circulation. On binding to bile acids or their conjugates, FXR induces in the liver the expression of the canalicular transporters BSEP, multidrug resistance protein 3 (MDR3; *ABCB4*), and multidrug resistance-related protein 2 (MRP2; *ABCC2*), while it represses the basolateral sodium taurocholate cotransporting polypeptide (NTCP; *SLC10A1*). In addition, FXR also inhibits transcriptional activity of cholesterol 7α-hydroxylase (CYP7A1), the rate limiting step in the conversion of cholesterol to bile acids. In the intestine, FXR positively regulates the ileal bile acid binding protein (I-BABP) and down-regulates the apical sodium-dependent bile salt transporter (ASBT; *SLC10A2*).

salts therefore can be invoked to explain liver injury and cholestasis in this disease. The most common BSEP mutations detected in affected individuals have been found to prevent protein from trafficking to the canalicular membrane of the hepatocyte.[42] Consequently, a complete absence of BSEP canalicular expression has usually been found in liver biopsies from these patients.[43] Therefore, in most cases, immunohistochemical staining for BSEP can be used as a test to diagnose BSEP disease. FIC1 disease, initially described as "Byler disease," arises from mutations in *ATP8B1* gene, which is expressed in a wide range of tissues and encodes a P-type ATPase (FIC1, for "familial intrahepatic cholestasis 1") potentially involved in translocation of aminophospholipids from the outer to the inner leaflet of the plasma membrane bilayer.[44,45] How dysfunction of FIC1 leads to cholestasis remains uncertain, although recent findings have suggested downregulation of Farnesoid X receptor (FXR, *NR1H4*) as a possible mechanism.[46,47] FXR is a nuclear receptor highly expressed in the enterohepatic system, where it acts as a bile acid sensor that maintains a regular bile salt pool size and protects the hepatocyte from excessive intracellular bile acid concentration.[48,49] This is largely achieved through the regulation of the expression of specific transporters that drive enterohepatic circulation, including BSEP and the ileal apical bile salt transporter (ASBT), as well as key enzymes involved in bile acid biosynthesis and conjugation (Figure 2). Downregulation of FXR with "domino" effects on other genes has been reported to take place in both the liver and the intestine of FIC1 disease patients.[46,47] Consistent with this, *Fxr* null mice exhibit a phenotype similar to both FIC1 and BSEP diseases, with hypercholanemia, impaired secretion of bile

acids, and failure to thrive.[50] Indeed, many alterations in bile acid transport and metabolism can be now understood as a FXR-mediated action, and it is anticipated that a putative involvement of this or other bile acid-activated transcription factor in the pathogenesis of some idiopathic cholestatic liver diseases will be under study. In FIC1 disease, the use of FXR agonists could have potential therapeutic implications.

Treatments for BSEP disease and FIC1 disease have included ursodeoxycholic acid therapy, partial external biliary diversion, ileal exclusion, and liver transplantation. Partial biliary diversion has been reported to be more effective than ileal exclusion for the management of patients with PFIC.[51] It has also been shown that, following biliary diversion, hepatic ultrastructural appearance, bile acid composition, and biliary excretion improve in PFIC patients.[52] However, this procedure might not work equally well in the different PFIC subtypes. A recent study conducted in a large group of children with genetically documented FIC1 disease and BSEP disease has revealed that the outcome of biliary diversion is better in patients with BSEP disease; in FIC1 disease patients, response to this intervention is poor.[53] Interestingly, the outcome of partial biliary diversion in BSEP patients appears to depend on mutations found.[53]

BSEP disease is readily corrected by liver transplantation. In contrast, a number of clinical problems persist or arise in some patients with FIC1 disease after liver replacement, including pancreatitis and intractable diarrhea.[36,39] No catch-up in growth and development of steatosis in allograft have been also reported.[54] Some of these complications could be ascribed to persistent alterations in ileal bile acid transport owing to FXR downregulation, with increased presentation of bile salt to the ileum after liver transplantation leading to exacerbation of diarrhea. In this scenario, and taking into account the predominant role of FIC1 in the regulation of intestinal bile acid absorption,[55] it is suggested that ileal bypass procedure might be an efficacious alternative approach for the management of FIC1 disease.

Acknowledgment

References

1. Balistreri WF, Grand R, Hoofnagle JH, et al. Biliary atresia: current concepts and research directions. Summary of a symposium. Hepatology 1996;23:1682-92.
2. Lopez-Santamaria M, Gamez M, Murcia J, et al. Long-term follow-up of patients with biliary atresia successfully treated with hepatic portoenterostomy. The importance of sequential treatment. Pediatr Surg Int 1998;13:327-30.

3. Sokol RJ, Cara M, Narkewicz MR, et al. Pathogenesis and outcome of biliary atresia: current concepts. J Pediatr Gastroenterol Nutr 2003;37:4-21.

4. Desmet VJ. Congenital diseases of intrahepatic bile ducts: variations on the theme "ductal plate malformation." Hepatology 1992;15:1069-83.

5. Mazzioti MV, Willis LK Heuckeroth RO, et al. Anomalous development of hepatobiliary system in the *Inv* mouse. Hepatology 1999;30:372-8.

6. Schon P, Tsuchiya K, Lenoir D, et al. Identification, genomic organization, chromosomal mapping and mutation analysis of the human INV gene, the ortholog of a murine gene implicated in left-right axis development and biliary atresia. Hum Genet 2002;110:157-65.

7. Jacquemin E, Cresteil D, Raynaud N, et al. CFC1 gene mutation and biliary atresia with polysplenia syndrome. J Pediatr Gastroenterol Nutr 2002;34:326-7.

8. Bamford RN, Roessler E, Burdine RD, et al. Loss-of-function mutations in the EGF-CFC gene CFC1 are associated with human left–right laterality defects. Nat Genet 2000;26:365-9.

9. Zhang DY, Sabla G, Shivakumar P, et al. Coordinate expression of regulatory genes differentiates embryonic and perinatal forms of biliary atresia. Hepatology 2004;39:954-62.

10. Khan E. Biliary atresia revisited. Pediatr Dev Pathol 2004;7:109-24.

11. Perlmutter DH, Shepherd RW. Extrahepatic biliary atresia: a disease or a phenotype? Hepatology 2002;35:1297-304.

12. Bill AH, Haas JE, Foster GL. Biliary atresia: histopathologic observations and reflections upon its natural history. J Pediatr Surg 1977;12:977-82.

13. Ohya T, Fujimoto T, Shimomura H, et al. Degeneration of intrahepatic bile duct with lymphocyte infiltration into biliary epithelial cells in biliary atresia. J Pediatr Surg 1995;30:515-8.

14. Davenport M, Gonde C, Redkar R, et al. Immunohistochemistry of the liver and biliary tree in extrahepatic biliary atresia. J Pediatr Surg 2001;36:1017-25.

15. Ahmed AF, Otan H, Nio M, et al. CD8+ T cells infiltrating into bile ducts in biliary atresia do not appear to function as cytotoxic T cells. J Pathol 2001;193:383-9.

16. Bezerra JA, Tiao G, Ryckman FC, et al. Genetic induction of proinflammatory immunity in children with biliary atresia. Lancet 2002;360:1653-9.

17. Mack CL, Tucker RM, Sokol RJ, et al. Biliary atresia is associated with CD4+ Th1 cell-mediated portal tract inflammation. Pediatr Res 2004;56:79-87.

18. Shivakumar P, Campbell KM, Sabla GE, et al. Obstruction of extrahepatic bile ducts by lymphocytes is regulated by IFN-γ in experimental biliary atresia. J Clin Invest 2004;114:322-9.

19. Piccoli DA, Spinner NB. Alagille syndrome and the Jagged1 gene. Semin Liver Dis 2001;21:525-34.

20. Alagille E, Estrada A, Hadchouel M, et al. Syndromic paucity of interlobular bile ducts (Alagille syndrome of arteriohepatic dysplasia). Review of 80 cases. J Pediatr 1987;109:195-200.

21. Hoffenberg EJ, Narkewicz MR, Sondheimer JM, et al. Outcome of syndromic paucity of interlobular bile ducts (Alagille syndrome) with onset of cholestasis in infancy. J Pediatr 1995;127:220-4.

22. Emerick KM, Rand EB, Goldmuntz E, et al. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. Hepatology 1999;29:822-9.

23. Oda T, Elkahloun AG, Pike BL, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet 1997;16:235-42.

24. Li L, Krantz ID, Deng Y, et al. Alagille syndrome is caused by mutations in Jagged1, which encodes a ligand for Notch1. Nat Genet 1997;16:243-51.

25. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770-6.

26. Lemaigre FP. Development of the biliary tract. Mech Dev 2003;120:81-7.

27. Kodama Y, Hijikata M, Kageyama R, et al. The role of Notch signaling in the development of intrahepatic bile ducts. Gastroenterology 2004;127:1775-86.

28. Flynn DM, Nijjar S, Hubscher SG, et al. The role of Notch receptor expression in bile duct development and disease. J Pathol 2004;204:55-64.

29. Mumm JS, Kopan R. Notch signaling: from the outside in. Dev Biol 2000;228:151-65.

30. Gridley T. Notch signaling and inherited disease syndromes. Hum Mol Genet 2003;12:9-13.

31. Jones EA, Clement-Jones M, Wilson DI. JAGGED1 expression in human embryos: correlation with the Alagille syndrome phenotype. J Med Genet 2000;37:658-62.

32. Crosnier C, Attie-Bitach T, Encha-Razavi F, et al. JAGGED1 gene expression during human embryogenesis elucidates the wide phenotypic spectrum of Alagille syndrome. Hepatology 2000;32:574-81.

33. Kamath BM, Krantz ID, Spinner NB, et al. Monozygotic twins with a severe form of Alagille syndrome and phenotypic discordance. Am J Med Genet 2002;112:194-7.

34. Xue Y, Gao X, Lindsell CE, et al. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged 1. Hum Mol Genet 1999;8:723-30.

35. McCright B, Lozier J, Gridley T. A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency. Development 2002;129:1075-82.

36. Shneider BL. Progressive intrahepatic cholestasis: mechanisms, diagnosis and therapy. Pediatr Transplant 2004;8:609-12.

37. Whitington PF, Freese DK, Alonso EM, et al. Clinical and biochemical findings in progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr 1994;18:124-41.

38. Thompson R, Jansen PLM. Genetic defects in hepatocanalicular transport. Semin Liver Dis 2000;20:365-72.

39. Knisely AS. Progressive familial intrahepatic cholestasis: an update. Pediatr Dev Pathol 2004;7:309-14.

40. Strautnieks SS, Bull LN, Knisely AS, et al. A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998;20:233-8.

41. Thompson R, Strautnieks S. BSEP: function and role in progressive familial intrahepatic cholestasis. Semin Liver Dis 2001;21:545-50.

42. Wang L, Soroka CJ, Boyer JL. The role of bile salt export pump mutations in progressive familial intrahepatic cholestasis type II. J Clin Invest 2002;110:965-72.

43. Jansen PLM, Strautnieks SS, Jacquemin E, et al. Hepatocanalicular bile salt export pump deficiency in patients with progressive familial intrahepatic cholestasis. Gastroenterology 1999;117:1370-9.

44. Bull LN, van Eijk MJ, Pawlikowska L, et al. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet 1998;18:219-24.

45. Klomp LWJ, Vargas JC, van Mil SWC, et al. Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology 2004;40:27-38.

46. Chen F, Ananthanarayanan M, Emre S, et al. Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity. Gastroenterology 2004;126:756-64.

47. Alvarez L, Jara P, Sánchez-Sabaté E, et al. Reduced hepatic expression of farnesoid X receptor in hereditary cholestasis associated to mutation in ATP8B1. Hum Mol Genet 2004;13:2451-60.

48. Chawla A, Saez E, Evans RM. Don't know much bile-ology. Cell 2000;103:1-4.

49. Chiang JY. Bile acid regulation of gene expression: roles of nuclear hormone receptors. Endocr Rev 2002;23:443-63.

50. Sinal CJ, Tohkin MM, Miyata M, et al. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 2000;102:731-44.

51. Kalicinski PJ, Ismail H, Jankowska I, et al. Surgical treatment of progressive intrahepatic cholestasis: comparison of partial biliary diversion and ileal bypass. Eur J Pediatr Surg 2003;13:307-11.

52. Kurbegov AC, Setchell KD, Haas JE, et al. Biliary diversion for progressive familial intrahepatic cholestasis: improved liver morphology and bile acid profile. Gastroenterology 2003;125:1227-34.

53. Thompson RJ. Surgical management of PFIC. Abstracts of the 2nd World Congress of Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 39:2004 (abstr P0019).

54. Lykavieris P, van Mil S, Cresteil D, et al. Progressive familial intrahepatic cholestasis type 1 and extrahepatic features: no catch-up of stature growth, exacerbation of diarrhea, and appearance of liver steatosis after liver transplantation. J Hepatol 2003;39:447-52.

55. Pawlikowska L, Groen A, Eppens EF, et al. A mouse genetic model for familial cholestasis caused by ATP8B1 mutations reveals perturbed bile salt homeostasis but no impairment in bile secretion. Hum Mol Genet 2004;13:881-92.