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Liver diseases related to *MDR3* (*ABCB4*) gene deficiency

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1. ABSTRACT

Class III multidrug resistance P-glycoproteins, mdr2 in mice and MDR3 in human, are canalicular phospholipid translocators involved in biliary phospholipid (phosphatidylcholine) excretion.The role of a *MDR3* (*ABCB4*) gene defect in liver disease has been initially proven in a subtype of progressive familial intrahepatic cholestasis called PFIC3, a severe pediatric liver disease that may require liver transplantation.Several *MDR3* mutations have been identified in children with PFIC3 and are associated to low level of phospholipids in bile leading to high biliary cholesterol saturation index.MDR3 mutations are associated to loss of canalicular MDR3 protein and /or to loss of protein function.There is evidence that biallelic or monoallelic *MDR3* defect causes or predisposes to 6 human liver diseases (PFIC3, adult biliary cirrhosis, low phospholipid associated cholelithiasis syndrome, transient neonatal cholestasis, intrahepatic cholestasis of pregnancy, drug induced cholestasis).Some patients with MDR3 deficiency may benefit from ursodeoxycholic acid therapy and could be good candidates to a targeted pharmacological approach and/or to cell therapy in the future.

2. INTRODUCTION

The role of a defect of the multidrug resistance 3 gene (*MDR3*) in liver disease has been initially suspected in a subtype of progressive familial intrahepatic cholestasis (PFIC) (1).In a general sense, PFIC is an heterogeneous group of autosomal recessive liver disorders of childhood in which cholestasis of hepatocellular origin often presents in the neonatal period or the first year of life and leads to death from liver failure at ages ranging from infancy to adolescence (2-7).Recent molecular and genetic studies have allowed the identification of genes responsible for three types of PFIC and have shown that PFIC was related to mutations in hepatocellular transport system genes involved in bile formation (8-12).These findings now provide specific tools for the precise diagnosis of PFIC.The first type of PFIC, PFIC1, is caused by mutation of the *ATP8B1* gene (13, 14).This gene codes for a P-type ATPase (FIC1) which is expressed in several organs but whose function is not precisely known.The second type of PFIC, PFIC2, is caused by mutation of the *ABCB11* gene that codes for the ATP-dependent canalicular bile salt export pump (BSEP) (15-18).Despite cholestasis, patients with PFIC1 and PFIC2 have normal serum gamma-

glutamyltransferase (GGT) activity (4, 12, 16, 19, 20).Liver histology is characterized by the absence of a true ductular proliferation with only periportal biliary metaplasia of hepatocytes (19, 21).PFIC1 and PFIC2 will be considered in details in other chapters of this issue.The third type of PFIC, PFIC3, is caused by mutation of the *MDR3* (*ABCB4*) gene (1, 22-29).*ABCB4* codes for a P-glycoprotein of class III multidrug resistance, involved in biliary phospholipid excretion.PFIC3, can be distinguished from the other types by a high serum GGT activity and liver histology which shows ductular proliferation and inflammatory infiltrate in the early stages despite patency of intra and extrahepatic bile ducts (1, 22-29).The histological pattern occurring in this disorder is very similar to the hepatic injury observed in mice with a homozygous disruption of the *mdr2* gene (*mdr2 -/-* mice), the murine orthologue of *MDR3* (30).Mdr2 in mice and MDR3 in human, are phospholipid translocators involved in biliary phospholipid (phosphatidylcholine) excretion and are predominantly, if not exclusively, expressed in the canalicular membrane of the hepatocyte (26, 28, 31-34).An abnormal expression of the *MDR3* gene (low amount of liver mRNA) and low biliary phospholipid concentration have been found in PFIC3 patients (1, 29).MDR3 P-glycoprotein belongs to the family of ATP binding cassette (ABC) transporters and the *MDR3* gene is localized on chromosome 7q21.This review article will consider the pathophysiology of genetic *mdr2/MDR3* deficiency and the different pediatric and adult cholestatic diseases which are now known to be caused by a genetic *MDR3* defect.So far, there is evidence that biallelic or monoallelic *MDR3* defect causes or predisposes to 6 human liver diseases (PFIC3, adult biliary cirrhosis, low phospholipid associated cholelithiasis syndrome, intrahepatic cholestasis of pregnancy, transient neonatal cholestasis, drug induced cholestasis) (26, 28).Finally, the therapeutic options, including drug therapy that may target MDR3 expression and function, will be considered.

3. THE *Mdr2* KNOCKOUT MOUSE : A MODEL OF LIVER PATHOLOGY DEFICIENT IN BILIARY PHOSPHOLIPID SECRETION

Mdr2 (-/-) mice suffer from liver disease that starts at a few weeks of age and progresses throughout life (30, 35).The most histologic striking feature, besides hepatocyte necrosis and dilated canaliculi, is the presence of a cholangiopathy represented by portal tract inflammation and a severe ductular proliferation which progress through the first 3 months of age.At the age of 4 to 6 months, the *mdr2* (-/-) mice start to develop nodules in the liver parenchyma, which histologically resemble the picture of chemically induced hepatocarcinogenesis.These nodules develop in hepatocellular carcinoma, and in mice older than 1 year metastases were observed in the lungs.The mechanism of tumor formation is unclear.Hepatocyte replication as a consequence of cell damage could be involved in tumor formation but this does not hold for cholangiocytes which proliferate even stronger.The absence of mdr2 P-glycoprotein could lead to accumulation of carcinogenic compounds in the hepatocytes.This could explain that tumors seem to be derived from hepatocytes rather than from cholangiocytes.An important observation from this model is that the bile of *mdr2* (-/-) mice is almost devoided of phosphatidylcholine whereas bile salt secretion is normal.This suggested that mdr2 P-glycoprotein was involved in the biliary secretion of phosphatidylcholine.In this model, the absence of mdr2 P-glycoprotein function clearly has deleterious effects on the bile canaliculi and the biliary cells.The cholangiopathy is probably caused by the cytotoxicity of bile salts in absence of biliary phospholipids.The cholangiopathy observed in the *mdr2* (-/-) mice may be relatively mild because of the relatively hydrophilic bile salt composition in this animal.Increasing the hydrophobicity of the bile salt pool by cholate feeding leads to a more severe liver pathology, whereas further decreasing its hydrophobicity by ursodeoxycholate acid (UDCA) feeding improves liver histology (36).Upon cholate feeding, bile cholesterol crystals have been observed in mdr2 (-/-) mice.Because the human bile salt pool is much more hydrophobic than in mice, it was expected that a defect of biliary phospholipid secretion in human could have much more dramatic consequences than in the mouse.Nevertheless, *mdr2* (-/-) mice develop sclerosing cholangitis, while sclerosing cholangitis like cholangiopathy has not been described in PFIC3 patients (29, 37, 38).Currently, there is no evidence for a role of *MDR3* variants as modifier gene in sclerosing cholangitis in adults (39-43).The production of this mouse model with a specific defect in biliary phospholipid secretion has made possible the identification of the analogous inherited human liver disease called PFIC3 and due to MDR3 deficiency (1, 22, 29).

4. THE SPECTRUM OF LIVER DISEASES RELATED TO *MDR3* DEFICIENCY

4.1.Progressive familial intrahepatic cholestasis type 3 (PFIC3)

4.1.1.PFIC3 phenotype

The disease transmission is autosomal recessive.Parents of affected patients are frequently related and a similar liver disease is often observed in siblings.Age at first symptoms ranges from 1 month to 20.5 years of age.First symptoms in most cases consist of the presence of jaundice, discoloured stools, hepatomegaly, splenomegaly or pruritus.Clinical signs of cholestasis are noted within the first year of life in about one third of patients and rarely in the first month (neonatal) period.Gastrointestinal bleeding due to portal hypertension and cirrhosis is the presenting symptom in adolescent or young adult patients.Initial serum liver tests (mean) show: elevated alanine aminotransaminase activity (5xN), conjugated bilirubin concentration (2xN), alkaline phosphatase activity (2xN), GGT activity (13xN), total bile acid concentration (25xN), and normal cholesterol concentration and prothrombin time.Evolution is characterized by chronic icteric or anicteric cholestasis, portal hypertension and liver failure.In half of the patient, liver transplantation is required at a mean age of 7.5 years.No liver tumor has been reported.Liver histology obtained at time of diagnosis shows portal fibrosis and ductular proliferation with mixed inflammatory infiltrate.In a few instances cholestasis is present in the lobule and there is giant transformation of hepatocytes.Cytokeratin immunostaining confirms the

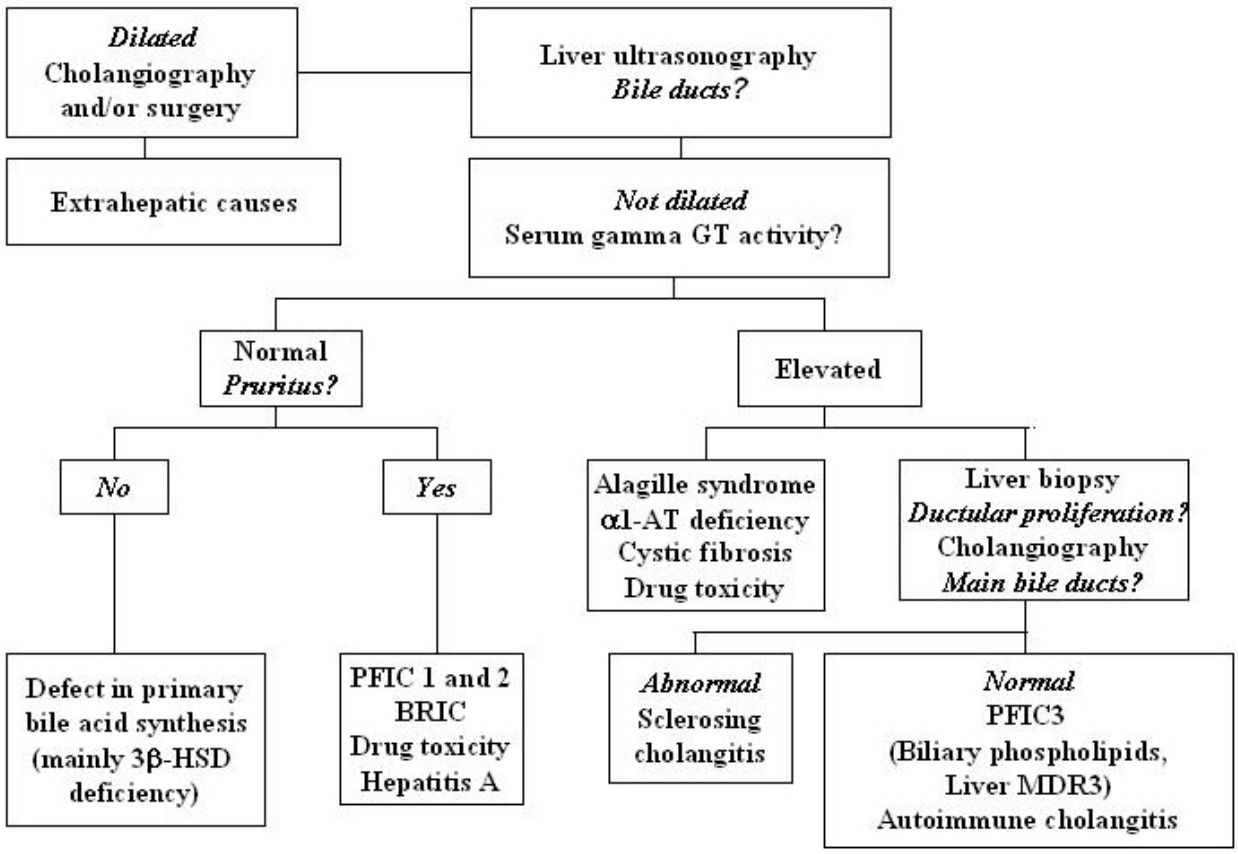

Figure 1. A schematic approach to the etiology of cholestasis in children, excluding the neonatal period (*In neonates, biliary atresia is the main cause of cholestasis*).PFIC, progressive familial intrahepatic cholestasis, 3ß-HSD deficiency, 3ß-Hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency; MDR3, multidrug resistance 3.

strong ductular proliferation within the portal tract (22).At a later stage, there is extensive portal fibrosis and a typical picture of biliary cirrhosis.Interlobular bile ducts are seen in most portal tracts, and there is neither periductal fibrosis nor biliary epithelium injury.In a few instances cholestasis is present in the lobule and in some ductules containing bile plugs.Cholangiography is normal and ultrasonography of the liver shows normal bile ducts in all patients (1, 22, 29).These results allow to distinguish PFIC3 patients from those with sclerosing cholangitis on the basis of histological and cholangiographic data.They can also be distinguished from patients with the other types of PFIC (PFIC1 and PFIC2) in that they present very rarely with cholestatic jaundice at the neonatal period, but rather in late infancy, childhood or in young adulthood (4, 9, 10, 16, 18, 29).Patients with PFIC3 have a persistent high serum GGT activity, moderately raised concentrations of serum primary bile salts and a mild pruritus. PFIC3 carries a higher risk of portal hypertension and gastrointestinal bleeding and ends in liver failure at a later age but as patients with PFIC1 and PFIC2 phenotypes, most of them have cholesterol level within the normal range.This may be explained by the inability of PFIC3 patients to generate lipoprotein X, the formation of which is mediated by class III P-glycoproteins (44).Indeed, we found no lipoprotein X in the serum of patients harboring homozygous nonsense *MDR3* mutations (29).A schematic approach to the diagnosis of PFIC3 is proposed in Figure 1. This combined clinical, biochemical, histological and radiological approach associated to biliary phospholipid dosage, and liver MDR3 immunostaining should help to select PFIC3 candidates in whom a molecular diagnosis of *MDR3* deficiency could be proposed.Children with PFIC3 are at risk to develop intrahepatic and extrahepatic cholesterol cholelithiasis and drug induced cholestasis (DIC) (26).Girls under UDCA therapy who reach adulthood with their native liver are at risk to develop severe intrahepatic cholestasis of pregnancy and must not stop UDCA during pregnancy (24, personal communication, Emmanuel Jacquemin, Nathalie Ganne-Carrié, Hôpital Jean Verdier, Bondy, France).

4.1.2.Biliary lipids

In PFIC3 patients, the biliary phospholipid level is dramatically decreased (1-15 % of total biliary lipids; N = 19-24%) despite the presence of normal concentration of bile salts in bile.Such finding is in favour of MDR3 deficiency.Biliary bile salt to phospholipid and cholesterol to phospholipid ratios are approximately 5 fold higher than in wild type bile.The residual percentage of biliary phospholipids seems directly related to the severity of *MDR3* mutation and consequently to residual activity of

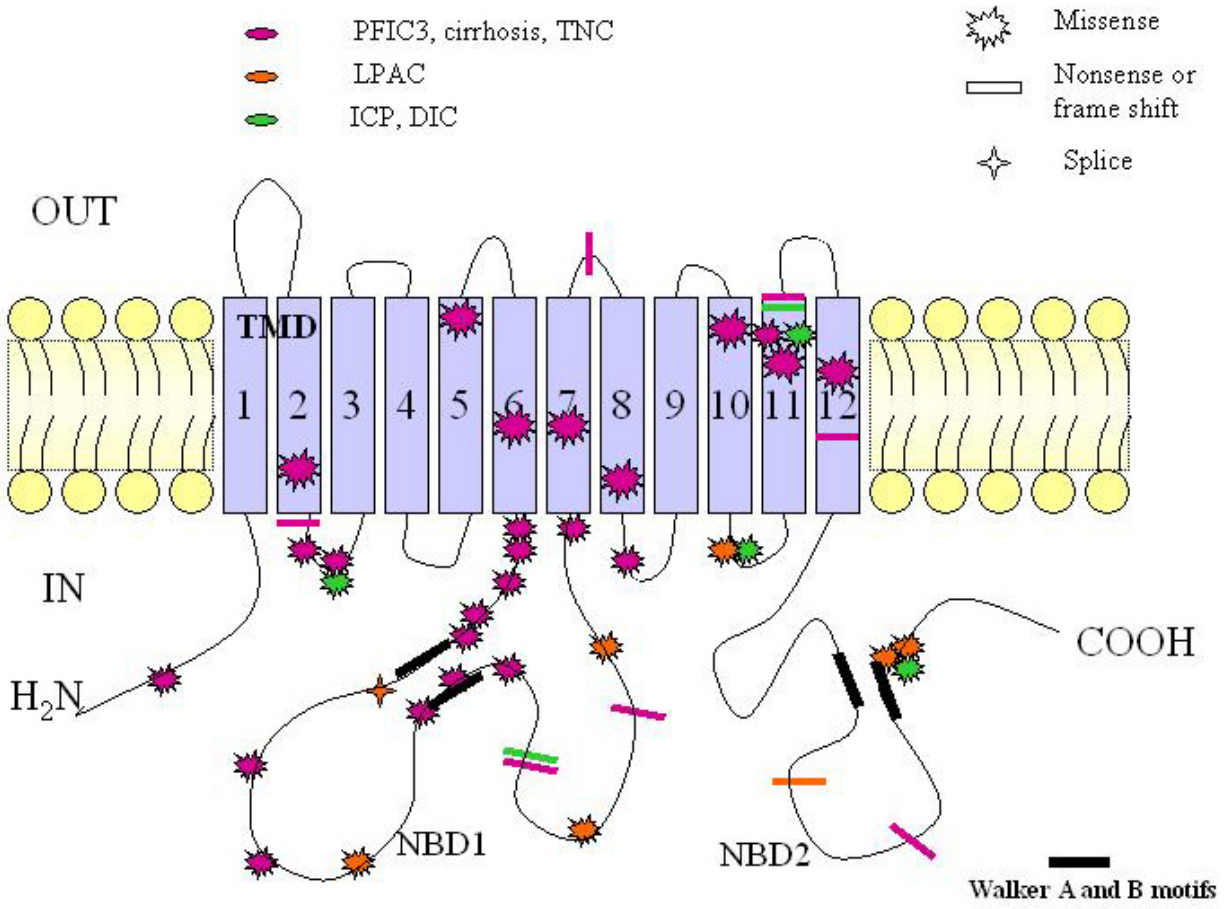

Figure 2. Schematic representation of MDR3 protein.Disease-associated mutations identified in patients from the pediatric and adult hepatology units of Bicêtre hospital are depicted.PFIC3, progressive familial intrahepatic cholestasis type 3; ICP, intrahepatic cholestasis of pregnancy ; TNC, transient neonatal cholestasis ; LPAC, low phospholipid associated cholelithiasis ; DIC, drug induced cholestasis.

MDR3 P-glycoprotein.Patients with "severe" mutation (nonsense, frameshift) have percentage of biliary phospholipids < 2%, while patients with missense mutations have percentage of biliary phospholipids ≥ 2%.In our experience, the threshold that predicts a positive answer to UDCA therapy, is represented by a percentage of biliary hospholipids of 7%.The normal concentration of biliary primary bile salts distinguish PFIC3 patients from those with PFIC1 and PFIC2 (6, 7, 16).

4.1.3. Liver MDR3 immunostaining

Patients with *MDR3* mutations have different extent of MDR3 P-glycoprotein canalicular immunostaining.Complete absence of canalicular staining is observed in patients with mutations leading to a truncated protein and in a few patients with missense mutations.A faint or normal MDR3 canalicular staining is only observed in patients with missense mutations (22, 25, 29).This means that a faint canalicular staining also suggests the existence of a MDR3 defect and that a normal canalicular staining does not exclude the presence of a MDR3 dysfunction.The combination of abnormal MDR3 canalicular immunostaining and low percentage of biliary phospholipids is highly suggestive for *MDR3* deficiency.

4.1.4.MDR3 mutations

In our experience, *MDR3* sequence analysis in 50 PFIC3 patients revealed around 30 different *MDR3* mutations (29, personal communication Emmanuel Jacquemin) (Figure 2).Mutations were characterized on both alleles in most cases.In one third of cases, mutations gave rise to a truncated protein.When tested, no MDR3 P-glycoprotein could be detected by immunostaining in the livers of these patients.The absence of MDR3 protein can be explained in two ways.The truncated protein may be broken down very rapidly after synthesis giving rise to extremely low steady state levels of the protein.More likely, the premature stop codon may lead to instability and decay of *MDR3* mRNA (45).This latter explanation is supported by the near absence of *MDR3* mRNA by northern blotting of livers of several patients (1).The two third remaining patients had missense mutations.Some of them were found in the highly conserved aminoacids sequences of the Walker A and B motifs which are involved in ATP-binding (46).Such aminoacid changes in

the Walker A or B motif are generally not compatible with ATPase activity and transport processes (46-49).Other missense mutations, were located in transmembrane domains and near the first Walker motifs.Site-directed mutagenetic analysis of P-glycoprotein has shown that mutations in transmembrane domains are important for substrate specificity and that mutations localized near Walker motifs may disrupt the function of the transporter (46, 50-52).Alternatively, missense mutations might result in intracellular misprocessing of MDR3 as shown for other ABC transporters (53-56).Indeed, such missense mutations were associated with a decreased level of MDR3 canalicular protein (29).Whatever the mechanism involved, the low level of biliary phospholipids found in patients with missense mutations demonstrates the MDR3 functional defect (29).Interestingly, we found evidence that some affected children had a missense mutation representing probably a polymorphism (R652G) (29).It may be that such aminoacid polymorphism has mild consequences, explaining the favourable outcome of patients with this mutation under UDCA therapy, or that in certain circumstances such as pregnancy, it leads to clinical symptoms (18, 26, 57-59).In human liver, this polymorphism is associated with low expression of MDR3.It has been shown for MDR1 P-glycoprotein that aminoacid polymorphism may affect the protein function (60).Ideally, it would be desirable to have a functional mean to distinguish disease mutations from normal variants (49, 56, 61, 62).In very rare patients (< 10%) with a PFIC3 phenotype, only one mutated allele or no mutation was identified.This can be explained by mutations that may map in regulatory sequences of the gene.A gene involved in *MDR3* transcription (i.e.*FXR*) or in protein trafficking could also be involved (63).It is also possible that other genes to be discovered and involved in bile formation may be responsible for PFIC3 phenotype.Furthermore, it may be hypothesized that combined heterozygous mutations for 2 genes (i.e.*MDR3* and *BSEP*) lead to PFIC3 like phenotype.An interesting possibility is also that in heterozygous state, the mutated protein may have a dominant negative effect on MDR3 expression/function (62).Heterozygosity of parents for the *MDR3* defects found in affected patients confirmed the recessive inheritance of the disease.This understanding has already allowed prenatal diagnosis (64).Other teams have subsequently reported on identification of MDR3 mutations in PFIC3 patients (23, 27).The use of a resequensing chip dedicated to genetic cholestasis could facilitate identification of *MDR3* mutation (65).

4.1.5.Genotype - phenotype correlation

In our experience, compared to children having a *MDR3* mutation leading to a truncated protein, children with a *MDR3* missense mutation have a less severe disease, with an onset later in life and a slower progression which could be favorably modified by chronic administration of UDCA in about 50% of cases (66).One can hypothesize that these differences are related to a residual transport activity in case of missense mutation.The fact that none of the patients with truncated protein responded to UDCA treatment is in line with this hypothesis (22).Response to UDCA in patients with missense mutation may be the result of residual transport activity, leading to residual phospholipid concentration in bile combined with enrichment of the bile salt pool with UDCA reducing bile salt toxicity below a critical threshold (24, 36).Alternatively, the effect of UDCA might be related to up-regulation of MDR3 P-glycoprotein expression since it has been shown that UDCA up-regulates mdr2 P-glycoprotein expression in primary hepatocytes (67).

4.1.6.Mechanism of liver pathology

Findings in PFIC3 patients confirm the functional homology between the mouse and human genes and further suggests that biliary phospholipid excretion is limited by the amount of mdr2 or MDR3 P-glycoproteins present at the canalicular membrane of the hepatocyte (22, 25, 29, 30, 33, 49, 68).The mechanism of liver damage in PFIC3 patients is likely related to the absence of biliary phospholipids (29).Injury to bile canaliculi and biliary epithelium results probably from continuous exposure to hydrophobic bile salts, the detergent effects of which are no longer countered by phospholipids leading to cholangitis (Figure 3) (26).In addition, the stability of mixed micelles in bile is determined by a three-phase system, in which a proper proportion of bile salts and phospholipids are necessary to maintain solubility of cholesterol.The absence of phospholipids would be expected to destabilize micelles and promote lithogenicity of bile with crystallization of cholesterol, which could favour small bile duct obstruction (Figure 4).These cholangiopathy mechanisms fit well with the histologic findings such as ductular proliferation and ductules containing bile plugs (22, 26, 29).Thus, PFIC3 represents an important example of hepatocellular (canalicular) transport defect that leads to the development of cholangiopathy.While *mdr2* (-/-) mice develop sclerosing cholangitis like disease, this type of cholangiopathy has not been reported in PFIC3 patients, and there is no evidence for a role of *MDR3* genetic variation in the pathogenesis of primary sclerosing cholangitis (28, 29, 37, 39, 42, 43).

4.2.Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is characterized by the occurrence of cholestasis during pregnancy in women with an otherwise normal medical history (69).ICP causes fetal distress, spontaneous premature delivery and unexplained third trimester intra uterine death.The classical maternal feature is generalized pruritus, becoming more severe with advancing gestation and abnormal serum liver tests.Maternal serum total bile salt concentration is raised compared to normal pregnancy and this is thought to be due to abnormal biliary transport across the canalicular membrane of hepatocyte.Usually serum GGT activity is within the normal range but in a subgroup of women it is increased (18, 53, 59, 69-76).Liver tests return to normal and pruritus disappears after delivery.Familial cases of ICP have been reported as well as cholestasis induced by oral contraceptive pill in non pregnant women who suffered previously of ICP or belonging to a family with a history of ICP (69, 77).This suggests that a genetic predisposition may exist in some cases of ICP.The link between PFIC3 and ICP has been established when it has been found, within the families of several different children with PFIC3, each child having a distinct nonsense or missense homozygous *MDR3*

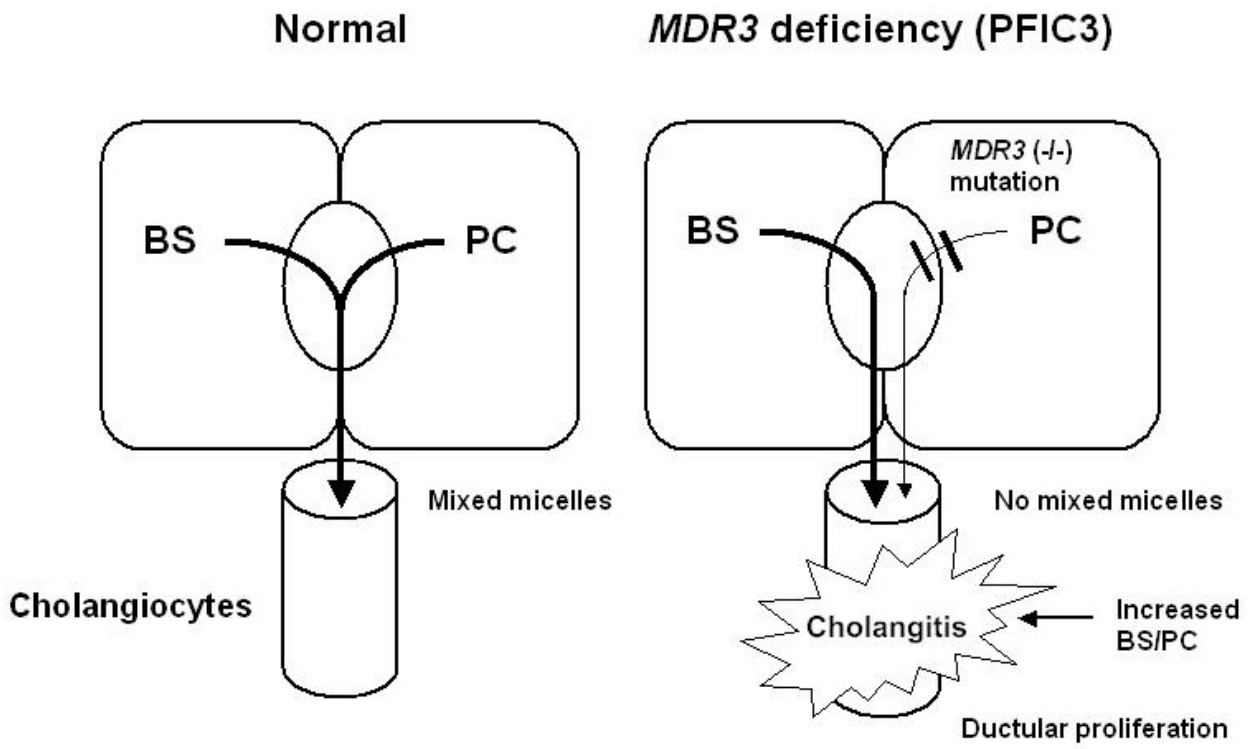

Figure 3. *MDR3* deficiency in progressive familial intrahepatic cholestasis type 3 (PFIC3).Left panel : Under normal conditions, phosphatidylcholine (PC) in bile protects cholangiocytes from bile salts (BS) toxicity by forming mixed micelles.Right panel : A mutation of the *MDR3* gene results in decreased biliary PC level and high BS to PC ratio and causes bile duct injury (cholangitis and ductular proliferation).These findings resemble hepatic injury in *mdr2* (-/-) mice.

mutation, that heterozygous women had experienced typical recurrent episodes of ICP (22, 29, 59).These familial observations of ICP with slightly elevated serum GGT activity provide arguments for a genetic basis of ICP and may explain the aspect of a dominant transmission trait reported previously (77).It is likely that the heterozygous state for a *MDR3* gene defect represents a genetic predisposition in these families, since cholestasis was not present in every pregnancy in these women (18, 59).Associated non genetic factors, such as female sex hormones and metabolites, could modify *MDR3* heterozygous state expressivity by decreasing normal allele expression (59).Indeed, a sterol responsive element exists in the *mdr2* gene promotor and could be involved in transcriptional control of mdr2 expression (63, 67, 78, 79).Alternatively, hormones could directly interact with MDR3 P-glycoprotein impairing its function (59, 80).Such events could favour the transient decompensation of the heterozygous state for a *MDR3* gene defect during pregnancy leading to ICP (18, 53, 59, 69-75b).As for PFIC3, cholestasis would result from the toxicity of bile in which detergent bile salts are not inactivated by phospholipids.While heterozygous *mdr2* (+/-) mice, with a maximal phospholipid secretion of 60% of controls do not develop liver disease, the appearance of liver injury in a heterozygous patient could be expected because in humans the bile salt pool is much more hydrophobic than in mice (30, 33, 35, 40, 81).This justifies to search for a *MDR3* gene mutation in ICP, particularly if serum GGT activity is high.Since the initial publication, numerous cases of ICP linked to a heterozygous *MDR3* defect, mostly with high serum GGT activity, have been reported in women with no known family history of PFIC, confirming that a *MDR3* defect represents a genetic predisposition to develop ICP (18, 53, 59, 69-75b).Furthermore, benign recurrent intrahepatic cholestasis (BRIC) and ICP have been reported in a single kindred suggesting that both cholestatic syndromes may be inter-related (82).This observation is very interesting because if usually in BRIC serum GGT activity is within the normal range, in a subgroup of women it is increased (83).All in all these findings suggest that in case of BRIC and/or ICP episodes with high serum GGT activity, a MDR3 defect should be considered (18, 53, 59, 70-75b).According to current classification of diseases related to *FIC1* or *BSEP* genes, ICP and BRIC related to *MDR3* deficiency should be reported under the terms ICP3 and BRIC3 (9, 11).It goes without saying that women who develop ICP due MDR3 deficiency should received UDCA therapy during the period of ICP, since UDCA has been proven efficient to reduce maternal and fetal complications of ICP (84, 85).

4.3.Cholesterol gallstone disease

Intrahepatic biliary lithiasis or gallbladder lithiasis have been found in children with PFIC3 and nonsense or missense *MDR3* mutations (29).Gallstones were also identified in some of their parents.Biliary lithiasis could be related to an increased biliary cholesterol

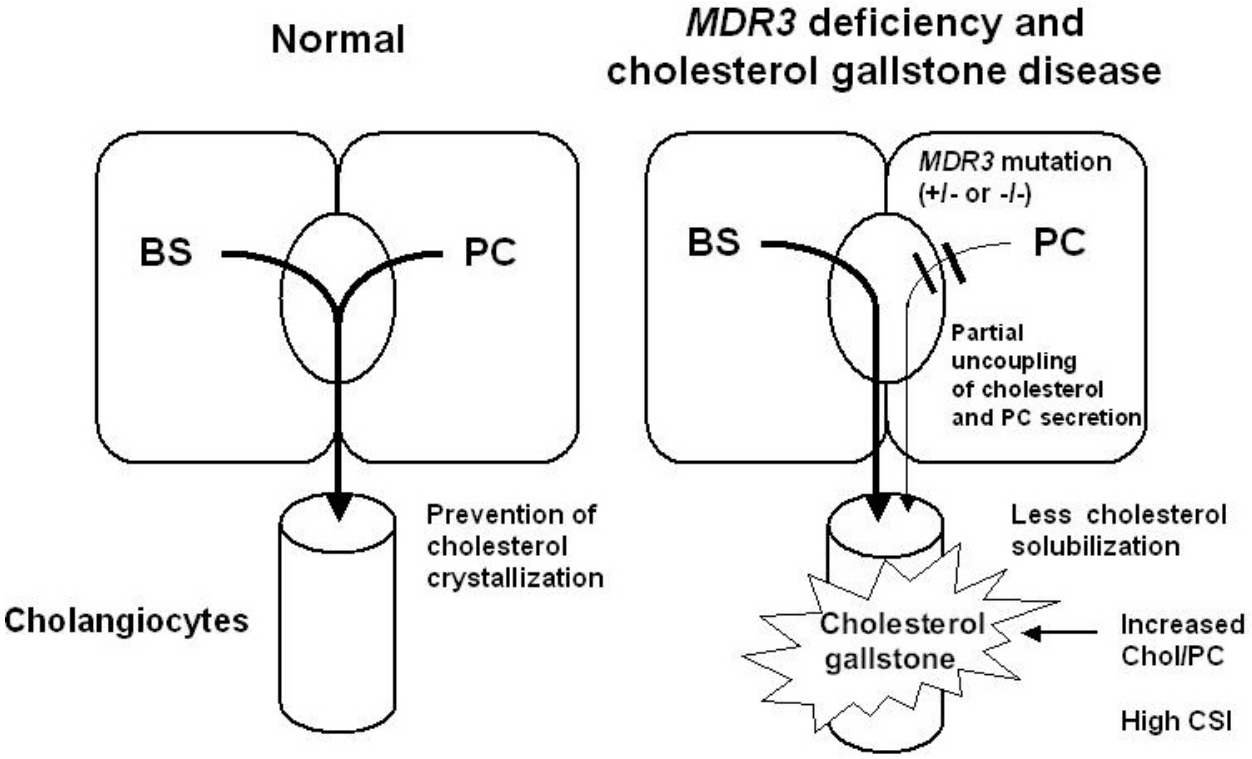

Figure 4. *MDR3* deficiency in cholesterol gallstone disease.Left panel : Under normal conditions, proper proportions of bile salts (BS) and phosphatidylcholine (PC) in bile are necessary to maintain solubility of cholesterol.Right panel : A mutation of the *MDR3* gene results in decreased biliary PC level and high cholesterol (Chol) to PC ratio leading to high biliary cholesterol saturation index (CSI).This will promote lithogenicity of bile with crystallization of cholesterol which could favour small bile duct obstruction.

to phospholipid ratio and suggests that, also in man, cholesterol and phospholipid biliary secretion can be partly uncoupled.This observation provides a mechanistic basis for gallstone formation (Figure 4).The absence or low level of phospholipids in bile would be expected to destabilize micelles and promote lithogenicity of bile with crystallization of cholesterol (81).This is very likely since cholesterol saturation index is abnormally increased in bile of PFIC3 patients (personal communication, Emmanuel Jacquemin).Indeed, many studies have reported ethnic and familial clusters of cholesterol gallstones suggesting genetic predisposition.Several studies performed in adult patients with symptomatic intrahepatic sludge, biliary microlithiasis and/or gallbladder cholesterol stones, have shown further evidence that *MDR3* mutations represent a genetic predisposition for cholesterol gallstone disease.This entity is reported under the term "Low Phospholipid Associated Cholelithiasis (LPAC)" syndrome (86-89).Mutations are heterozygous frame-shift, non sense or missense mutations or homozygous missense mutations and are mainly localized in important presumed protein domains.Also in these studies were biliary cholesterol to phospholipid ratio and cholesterol saturation index abnormally elevated.Of importance was also the observation that the symptomatology (mild cholestasis, biliary pain, pancreatitis or cholangitis) recurred after cholecystectomy and was dramatically enhanced during pregnancy or after starting oral contraception.Again, it is likely that the genetic predisposition represented by the MDR3 defect is decompensated by female sex hormones.Further recurrence is prevented by long term oral administration of UDCA.Cholecystectomy is only indicated in the case of symptomatic gallstones but not when only sludge is present in the gallbladder, because it usually disappears under UDCA therapy.Biliary drainage or partial hepatectomy may be indicated in case of symptomatic non cystic intrahepatic bile duct dilatations filled with gallstones (89).

4.4.Drug induced cholestasis

Several lines of evidence suggest that MDR3 deficiency could be involved in drug induced cholestasis (i.e., oral contraceptive pill induced cholestasis).First, MDR3 deficiency predisposes to ICP (see above).Secondly, cases of cholestasis induced by oral contraceptive pill in non pregnant women who suffered previously of ICP or belonging to a family with a history of ICP have been reported (77, 82).Third, in patients with cholesterol gallstone disease due to MDR3 deficiency, symptomatology was dramatically enhanced during pregnancy or after starting oral contraception (86, 87, 89).Fourth, a case of asymptomatic cirrhosis due to MDR3 deficiency in a young adult woman has been revealed after starting oral contraceptive pills (24).As we have already discussed in this review the genetic predisposition represented by nonsense or missense (including aminoacid

polymorphism) *MDR3* mutations could manifest under the pressure of xenobiotic intake (26, 57).A MDR3 defect should be searched in women who experienced cholestasis under oral contraception, especially if cholestasis is characterized by high serum GGT activity.It is postulated that xenobiotics that inhibit ABC proteins, and more specifically MDR P-glycoprotein, could induce cholestasis in predisposed patients with MDR3 deficiency (80).Recent data have clearly shown that some *MDR3* mutations are associated to the occurrence of drug induced cholestasis and therefore MDR3 deficiency represents very likely a genetic predisposition to develop drug induced cholestasis (58).

4.5.Transient neonatal cholestasis

This spontaneous resolving form of neonatal cholestasis, results from the association of several factors, including immaturity of bile secretion and perinatal disease leading to hepatic hypoxia or ischemia (90).In 10% of the children with transient neonatal cholestasis no remarkable events are identified during the neonatal period.It is obvious that transient neonatal cholestasis preferentially appears in children who have had perinatal suffering but it could also develop in predisposed children in absence of perinatal events (90).The predisposition could be represented by a heterozygous genetic defect in any hepatocellular canalicular ATP dependent transport system involved in bile formation (8, 11, 26).This could favour the transient decompensation of bile secretion processes which are underdeveloped in neonates (90).Since most of the children with transient neonatal cholestasis have high serum GGT activity when cholestatic, *MDR3* gene could be involved.Obviously, this speculative and provocative hypothesis needs to be proved in a large cohort of children, but we have already some evidence for it.Indeed, we have found (personal communication, Etienne Sokal, Cliniques St Luc, Brussels, Belgium, and Emmanuel Jacquemin) heterozygous MDR3 mutation in 3 children, without perinatal suffering, who had neonatal cholestasis with features compatible with PFIC3 (26, 29, 64, 91).In one of these children, the MDR3 P-glycoprotein function defect has been documented by low percentage of biliary phospholid and normal biliary bile salt level (29).In all children, clinical condition and liver tests normalized under UDCA therapy and remained normal after stopping UDCA.This evolution was similar to the one of transient neonatal cholestasis (90).These observations suggest that a heterozygous MDR3 missense mutation may be involved in some children with transient neonatal cholestasis.Furthermore, it is known that transient neonatal cholestasis does not develop in all neonates with perinatal distress.In this view, it might be that those neonates who develop transient neonatal cholestasis after perinatal suffering are in fact genetically predisposed (92).A genetic predisposition could precipitate the consequences of hypoxia-ischemia on ATP dependent canalicular mechanisms involved in bile secretion, leading to transient neonatal cholestasis (90).

4.6.Adult " idiopathic " biliary cirrhosis

A MDR3 defect was found in several young adults in whom a diagnosis of cirrhosis was made between the age of 13.5 and 20.5 years (24, 29).All had a missense mutation, have responded to UDCA therapy and are still alive with their native liver and under UDCA therapy at a mean age of 28 years (24, 29).Genotype-phenotype analysis has shown that *MDR3* gene mutation that leads to complete absence of function is lethal within the first decade of life unless liver transplantation is performed (29).By contrast, certain missense mutations that lead to residual activity (i.e., as expected in patients mentioned above) are probably spontaneously compatible with life until adolescence or early adulthood.For this reason, we believe that some young adults, or even older, with unexplained " idiopathic " cirrhosis of biliary type may have *MDR3* deficiency (26a, 26b).Indeed, a *MDR3* mutation has been detected in a woman who developed cholelithiasis in adolescence, followed by cholestasis of pregnancy and finally adulthood biliary cirrhosis at 47 years (93).

5.GENOTYPE – PHENOTYPE CORRELATION AMONG LIVER DISEASES RELATED TO *MDR3* DEFICIENCY

Schematically, tentative and coarse correlations between genotype, taking into account mutated allele number and mutation class, and initial phenotype can be established.Patients with PFIC3 and/or young adult biliary cirrhosis harbor biallelic mutations, compound heterozygous or homozygous, that are thought to have a serious deleterious effect on protein function in most cases (23, 24, 27, 29, 64).Other phenotypes mainly harbor heterozygous mutations, "severe" or "soft", or "soft" biallelic missense mutations (18, 24, 26, 29, 53, 58, 59, 64, 70-75b, 86-89, 93).Meanwhile, it is obvious that such correlations are difficult and fragile because a patient may develop several phenotypes during life (24, 93).

6.TREATMENT OF MDR3 DEFICIENCY (MAINLY PFIC3): PRESENT AND FUTURE

In our experience, oral administration of UDCA represents an alternative to liver transplantation in some children with PFIC3 (26, 29, 66).Indeed, around 30% and 15% of PFIC3 patients normalized or improved their liver tests under UDCA, respectively.All the 11 children previously reported and who responded to UDCA therapy are still alive with their native liver and under UDCA treatment, at ages ranging from 13 to 33 years (29).In patients with PFIC3 as in other types of cholestasis, the beneficial effect of UDCA may also be related to the modulation of biliary bile acid composition in favour of hydrophilic bile acids which might diminish cellular injury (29, 66).In the *mdr2 (-/-)* mouse model, feeding the noncytotoxic bile salt UDCA led to a complete replacement of the endogenous bile salt pool with UDCA and this halted the progression of the liver disease (36).It has been shown that nonresponders have a complete defect in phospholipid secretion (i.e., nonsense *MDR3* mutation) and it is likely that partial UDCA replacement is insufficient to reduce the increased bile salt toxicity in phospholipid-free bile of these patients (22, 29). Patients who do respond to UDCA therapy have a partial defect (e.g., missense *MDR3* mutation) and the residual phospholipid concentration in bile (threshold of 7% of

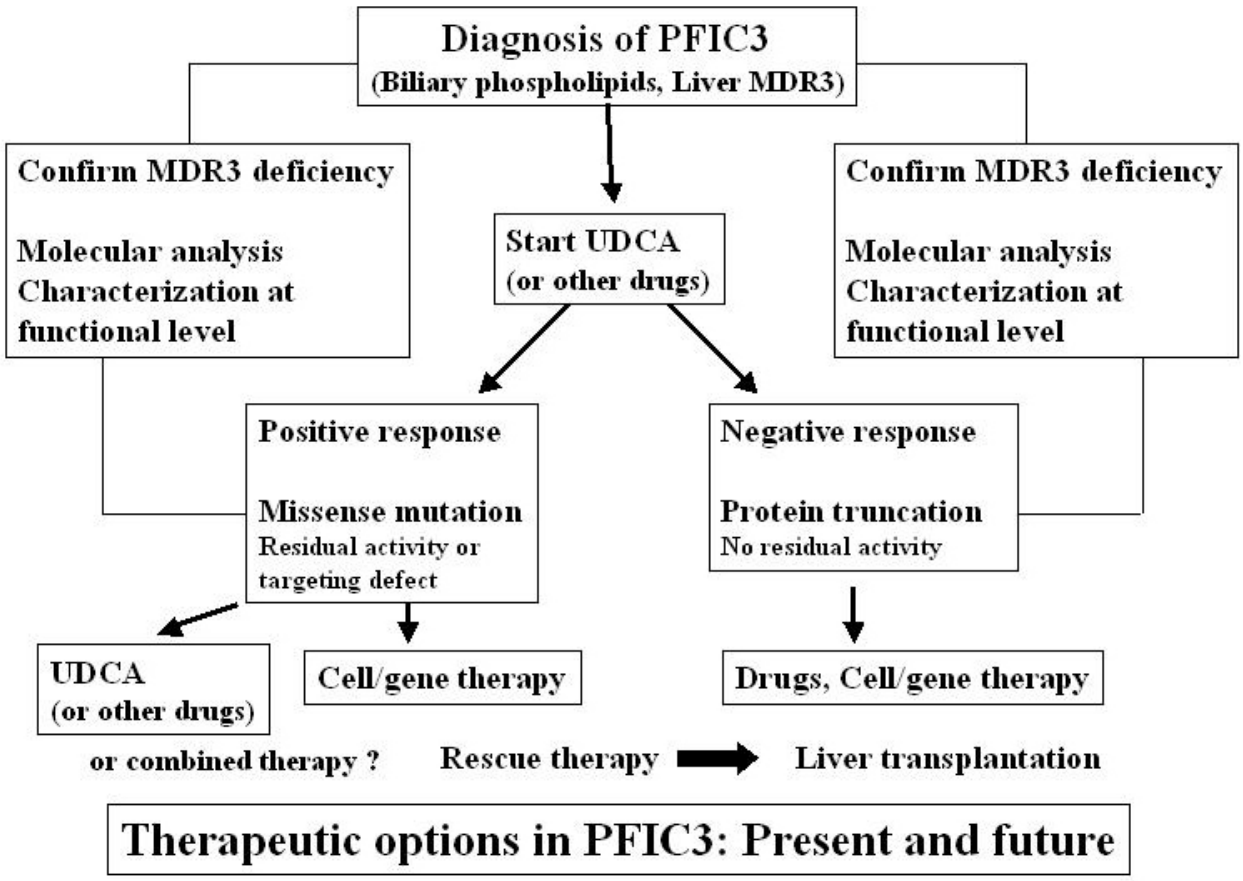

Figure 5. Therapeutic options in PFIC3 : Present and future.PFIC3, progressive familial intrahepatic cholestasis type 3; MDR3, multidrug resistance 3 ; UDCA, ursodeoxycholic acid.

total biliary lipids), combined with a partial UDCA replacement, may be sufficient to reduce the bile salt toxicity below a critical threshold (29).These correlations should help to select those PFIC3 patients who could benefit from UDCA therapy.It is our policy to consider UDCA in the initial therapeutic management of children with PFIC3.It could prevent evolution towards cirrhosis and therefore avoid, at least in the mean term, the need for liver transplantation in some children (29, 66).In case of failure with this therapy, liver transplantation still represents the treatment of choice.Furthermore, UDCA has been shown to be effective in the treatment of ICP and cholesterol gallstone disease (84-87, 89).In these conditions, UDCA should be the treatment of choice, especially if the disease causing mechanism is MDR3 deficiency.

New therapeutic options for MDR3 deficiency related diseases deserve also consideration and will certainly be tested in the future (Figure 5).First, a targeted pharmacological approach intended to induce MDR3 function is conceivable.Indeed several drugs (UDCA, statins, fibrates) are already known to up-regulate mdr2 P-glycoprotein expression in rat liver (67,78, 94, 95).MDR3 transcription seems mainly regulated by the nuclear receptor FXR (28, 63, 79, 96, 97).Drugs that could increase *MDR3* expression via FXR will be good candidates for PFIC3 therapy (97-100).It is possible that the beneficial effect of UDCA in PFIC3 patients is in part related to up-regulation of MDR3 P-glycoprotein expression (97, 98).It is hypothesized that patients who have a partial MDR3 defect with residual activity (i.e., missense *MDR3* mutation) might benefit from such treatments in the future (26, 28).A second pharmacological approach intended to overcome the arrest of translation induced by specific nonsense mutations (i.e., using aminoglycosides) or to retarget at the canalicular membrane a misfolded mutated protein blocked within the hepatocyte (i.e., using chaperone drugs) should also be considered (55, 56, 101-103).A third pharmacological approach, could be to induce another canalicular protein (i.e.MDR1 P-glycoprotein) that could functionally complement MDR3 P-glycoprotein (26).Experimental data exist that show that there is a kind of overlap substrate specificity between MDR1 and MDR3 proteins (80, 104).Such approach has already been tested fortuitously with success in one patient with cystic fibrosis.Indeed, a preliminary report has suggested that MDR1 could functionally complement CFTR (105, 106).While shown efficient in *mdr2* (-/-) mice, the effect of a lecithin rich diet has not been tested in children with PFIC3 (107).Finally, cell and gene therapies will certainly be considered in the

future.An elegant study performed in *mdr2* (-/-) mice has shown that the liver disease due to *mdr2* deficiency was corrected by transplantation of *mdr2* (+/+) or *MDR3* transgenic hepatocytes (108).It is expected that in PFIC3 normal transplanted human hepatocytes will profit from liver regeneration induced by the disease and that their proliferation will be selectively favoured by the fact that they protect themselves from bile salts.Consequently repopulation of the native liver by MDR3 expressing hepatocytes should be possible.This selective advantage should also apply to a gene therapy approach for PFIC3.When cell or gene therapy will be reasonably feasible in this liver disease, it will be probably necessary to propose it before the presence of severe liver fibrosis which might hamper liver repopulation by normal or genetically modified hepatocytes.In this view, it will be a new challenge for pediatricians and geneticians, who will have in a short time period, first, to identify children with PFIC3 and, second, to confirm and characterize (i.e.at the functional level) the MDR3 gene defect (26).Waiting for the molecular diagnosis of MDR3 deficiency, therapy with UDCA (or other drugs in the future) should be initiated in order to prevent liver damage.In case of mutation leading to protein truncation, no answer to UDCA is expected.In this situation, cell or gene therapy could be proposed.In presence of a mutation leading to residual activity (or to protein mistargeting) a positive response to UDCA (or other drugs) could be expected.In this situation the choice of therapeutic options between the pursuit of UDCA (or other drugs) and the decision to start cell/gene therapy, or a combination of drugs and cell/gene therapy, will need to be defined.The fact that liver transplantation will still be a rescue option in case of failure of these new treatments should facilitate their application to patients with PFIC3 and MDR3 deficiency.

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Abbreviations: mdr2, multidrug resistance 2; MDR3, multidrug resistance 3; PFIC, progressive familial intrahepatic cholestasis ; BSEP, bile salt export pump ; GGT, gamma-glutamyltransferase; UDCA, ursodeoxycholic acid ; BRIC, benign recurrent intrahepatic cholestasis ; ICP, intrahepatic cholestasis of pregnancy ; TNC, transient neonatal cholestasis ; LPAC, low phospholipid associated cholelithiasis ; DIC, drug induced cholestasis

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