Genetic cholestatic liver diseases : The example of progressive familial intrahepatic cholestasis and related disorders

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Introduction

One of the main features of pediatric hepatology is the importance of genetic disorders. Consequently, the development of new technologies for genomics and proteomics research should have an important impact on pediatric hepatology. These technological advances have already allowed the understanding of the role of genetic factors in the pathogenesis of some pediatric cholestatic liver diseases (1, table). It can be expected that it will also facilitate the development of novel treatments (e. g., gene, cell, and pharmacogenetics therapies) and molecular prenatal diagnosis. Characterization of genotype/phenotype correlations will further increase our ability to understand cellular and molecular disease relationships, to develop treatment strategies, and to improve counseling of families. In addition, it is very likely that many of the genetic discoveries that initially focus on pediatric liver disorders will have more widespread application to adult hepatobiliary physiology and diseases. One of the major advances in pediatric hepatology over the past 5 years has been the genetic and molecular characterization of the progressive familial intrahepatic cholestasis disorders.

Progressive familial intrahepatic cholestasis (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 usually ranging from infancy to adolescence (2). 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 (3-5). Liver diseases resembling PFIC that have recently been identified as inborn errors in primary bile acid synthesis represent distinct disorders (6). In our experience, PFIC represents 10 to 15% of causes of cholestasis in children, 10% of liver transplantation indications in children, and among PFIC, PFIC1 and PFIC2 represent 2/3 of cases and PFIC3 1/3 of cases. The true incidence of PFIC is not precisely known, but PFIC is considered a rare disease with an estimated incidence of 1/100000. Other liver diseases resembling PFIC are just being recognized. Amish hypercholanemia represents a PFIC like disorder not due to primary defect of transport system involved in bile formation but to defect of tight junction protein combined with a defect of primary bile acid conjugation (Table). Cholestasis is due low transport of unconjugated bile acids into bile and to bile leakage into plasma through abnormal canalicular tight junctions increasing paracellular permeability. Another category of progressive cholestastic liver disease of childhood could be due to abnormal villin expression leading to loss of structural integrity of canalicular microvilli impairing biliary secretion system function (Table). In the future, it will be interesting to see if these concepts of cholestasis due to defects of cytoskeletal or tight junction proteins may also apply for biliary diseases such as sclerosing cholangitis.

Progressive familial intrahepatic cholestasis type 1

The first type, called PFIC1 is caused by mutations in the *FIC1* gene (also designated *ATP8B1*) (4,7). In patients belonging to the Byler kindred, the disease is called Byler disease. This gene which encodes a P-type ATPase is located on human chromosome 18 and is also mutated in the milder phenotype, benign recurrent intrahepatic cholestasis (BRIC) and in Greenland familial cholestasis (4,7,8). The function of this P-type ATPase is unknown but it could be an aminophospholipid transporter responsible for maintening the enrichment of phosphatidylserine and phosphatidylethanolamine on the inner leaflet of the plasma membrane in comparison of the outer leaflet (4,9). How mutations in this protein cause cholestasis is unclear. FIC1 protein is located on the canalicular membrane of the hepatocyte (9,10). It is postulated that abnormal protein function might indirectly disturb the biliary secretion of bile acids,

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Table. — **Familial cholestatic liver diseases in children and adults : involved chromosomes and genes**

Disease	Chromosome	Gene	Reference n°
PFIC1, GFC, BRIC	18	*FIC1*	4
PFIC2	2	*BSEP*	3
PFIC3, ICP, DIC, CGD	7	*MDR3*	5
Inborn errors in primary bile	16	*3β-HSD (HSD3B7)*	6, 32
acid synthesis	7	*Δ4-3oxo-5βR (SRD5B1)*	33, 34
	8	*Oxysterol 7α OHase (CYP7B1)*	35
Aagenaes syndrome	15	?	36, 37
Amish hypercholanemia	9	*TJP2, BAAT, NTCP ?*	38, 39
NAICC	16	*Cirhin*	40
ARC syndrome	?	?	41
α1 antitrypsin deficiency	14	*α1 antitrypsin*	42
Alagille syndrome	20	*Jagged1*	1
Cystic fibrosis	7	*CFTR*	43
Sclerosing cholangitis (with ichthyosis)	3	?	44
Biliary atresia (with	2	*CFC1 ?*	45
polysplenia syndrome)	9	*INV ?*	46
Progressive cholestatic liver	2	*Villin ?*	47
disease of childhood	11	*Radixin ?*	48

PFIC, progressive familial intrahepatic cholestasis ; GFC, greenland familial cholestasis ; BRIC, benign recurrent intrahepatic cholestasis ; ICP, intrahepatic cholestasis of pregnancy ; DIC, drug-induced cholestasis ; CGD, cholesterol gallstone disease ; NAICC , north american indian childhood cirrhosis ; ARC, arthrogryposis - renal tubular dysfunction – cholestasis ; FIC1, familial intrahepatic cholestasis 1 ; BSEP, bile salt export pump ; MDR3, multidrug resistance 3 ; 3β-HSD, 3beta-hydroxy-C27-steroid-dehydrogenase isomerase ; Δ4-3oxo-5βR, delta4-3-oxosteroid 5beta reductase ; Oxysterol 7α OHase, oxysterol 7α hydroxylase ; TJP2, tight junction protein 2 ; BAAT, bile acid coenzyme A ; NTCP, sodium-taurocholate cotransporting polypeptide ; CFTR, Cystic fibrosis transmembrane conductance regulator ; CFC1, gene encoding the CRYPTIC protein ; INV, INV gene.

explaining the low biliary bile acid concentration found in PFIC1 patients (4,11). The *FIC1* gene is expressed in various organs, including the liver, pancreas, kidney and small intestine, but is more highly expressed in the small intestine than in the liver (7). Therefore, it is thought to also be involved in the enterohepatic cycling of bile salts. This may explain the chronic diarrhea present in a few children with PFIC1. This extrahepatic feature does not resolve after liver transplantation and may become intractable when biliary bile salt secretion is restored (4,12,13). Recent findings in FIC1 mutant mice are consistent with the fact that FIC1 is involved, directly or indirectly in the intestinal bile salt absorption (14). Another extrahepatic feature associated with PFIC1 is short stature, which does not improve after liver transplantation, suggesting a general cell biological function for FIC1 (4,12,13). It is very likely that FIC1 disease represents a continuum with intermediate phenotypes between the benign phenotype (BRIC) and the severe phenotype PFIC1 (4). So far, there is no good explanation of the phenotypic difference between patients with BRIC and those with PFIC1. Mutations analyses suggest that the mutations identified in patients with PFIC1 would severely disrupt protein function, whereas protein function would be only partially impaired in patients with BRIC. Genotype-phenotype associations will probably be complicated because dramatic variability in phenotypic presentation has already been identified in patients with BRIC with a common mutation. In addition many patients with FIC1 disease are compound heterozygous, which will also complicate the identification of genotype-phenotype correlations (4).

Progressive familial intrahepatic cholestasis type 2

The second type, called PFIC2 (previously called Byler syndrome and recently named BSEP deficiency) is caused by mutations in the *BSEP* gene (also designated *ABCB11*) (3,15). Byler syndrome has been defined as a disease similar to Byler disease but occuring in patients not belonging to the Byler family (2,11). The *BSEP* gene encodes the ATP-dependent canalicular bile salt export pump (BSEP) of human liver and is located on human chromosome 2. Mutations in this protein are responsible for the decreased biliary bile salt secretion described in affected patients, leading to decreased bile flow and accumulation of bile salts inside the hepatocyte with ongoing severe hepatocellular damage. So far, no clear genotype-phenotype correlation has been shown among PFIC2 patients, but it has been shown that children with *BSEP* mutations, regardless of the mutation type, had no canalicular BSEP protein expression (16). This suggests that immunohistochemical BSEP staining may be a useful diagnostic tool (3). Mice with a homozygous disruption of the *bsep* gene have a less severe phenotype than PFIC2 patients (17).

Phenotypic differences between PFIC1 and PFIC2

Patients with PFIC1 and PFIC2 have normal serum gamma-glutamyltransferase (GGT) activity, normal serum cholesterol level, high serum bile acid concentration and severe pruritus. Liver histology is characterized

by canalicular cholestasis and the absence of a true ductular proliferation with only periportal biliary metaplasia of hepatocytes (2). While phenotypic findings in PFIC1 and PFIC2 are similar, some slight phenotypic differences have been identified (2-5,11,12,16,18). In our experience, besides extrahepatic features, PFIC1 seems characterized in the first months of life by recurrent episodes of jaundice which become permanent later in the course of the disease. In PFIC2, initial presentation and evolution seem to be more severe, with permanent jaundice from the first months of life and rapid appearance of liver failure within the first years of life. In PFIC2, the liver architecture is more perturbed with lobular and portal fibrosis and inflammation, and a giant cell transformation which is more pronounced than in PFIC1 and seems to persist with time. These phenotypic differences have been also recently found in a small Taiwanese series and may be useful to differentiate FIC1 from BSEP-related disease (16). In this series, it was also shown that compared to PFIC1, PFIC2 patients had higher transaminase and alpha-fetoprotein levels (12,18). These biochemical differences between PFIC1 and PFIC2 may reflect the severe lobular injury present in PFIC2. These phenotypic features will be confirmed only when genotype-phenotype correlations will be available in large series.

Progressive familial intrahepatic cholestasis type 3

The third type of PFIC, called PFIC3 (recently named MDR3 deficiency), 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 with normal cholangiogram (5,19-21). We and others have reported in several patients that a genetic defect in the *MDR3* gene (also designated *ABCB4*) underlies PFIC3 which shares histological and biochemical (low biliary phospholipid level) features with mice with a homozygous disruption of the *mdr2* gene (5,19-23). The *MDR3* gene is located on chromosome 7. Class III Multidrug Resistance (MDR) P-glycoproteins (P-gp), 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 (5). Cholestasis would result from the toxicity of bile in which detergent bile salts are not inactivated by phospholipids, leading to bile canaliculi and biliary epithelium injuries. The absence of phospholipids in bile would be expected to destabilize micelles and promote lithogenicity of bile with crystallization of cholesterol, which could favor small bile duct obstruction. These cholangiopathy mechanisms fit well with the histologic findings such as ductular proliferation. PFIC3 represents an important example of canalicular transport defect that leads to the development of cholangiopathy. The phenotypic spectrum of PFIC3 ranges from neonatal cholestasis to cirrhosis in young adults. A recent series has suggested that by comparison to children with an MDR3 mutation leading to a truncated protein, children with an MDR3 missense mutation have less severe disease, with an onset later in life and a slow progression which is favorably modified by chronic administration of ursodeoxycholic acid (UDCA) (21). One can hypothesize that these differences are related to a residual transport activity in case of missense mutation. There is now strong evidence that in addition to PFIC3, an MDR3 defect can be involved in intrahepatic cholestasis of pregnancy and cholesterol gallstone disease (24-29). MDR3 defect is also likely to be involved in some cases of drug induced cholestasis, transient neonatal cholestasis and adult idiopathic cirrhosis (5,30).

Treatment of progressive familial intrahepatic cholestasis

Some patients with PFIC1 or 2 may benefit from oral administration of ursodeoxycholic acid (UDCA) and/or surgical partial external biliary diversion (or ileal exclusion) (2-4). If these therapies fail, liver transplantation represents the only alternative. So far, clear genotype-phenotype correlation data are missing and remain to be defined in order to identify those PFIC1-2 patients who could benefit from UDCA or biliary diversion. In our experience, extrahepatic features, such as diarrhea and short stature sometimes associated with PFIC1, do not improve or may be aggravated after successful biliary diversion or liver transplantation (12). These extrahepatic features might be favourably managed by bile adsorptive resin treatment (12,13). Patients with PFIC3 may also benefit from UDCA therapy, especially those with some missense mutations (21). Non-responders to UDCA are candidates for liver transplantation. In the future, therapies such as cell or gene therapies, might represent an alternative therapy for all types of PFIC (3-5,31). In addition, a specific pharmalogical approach intended to compensate for the protein dysfunction in genotypically selected patients deserves consideration (5). In this view, it will be a new challenge for pediatricians and geneticists, who first will have to identify children with PFIC1-3 and, second will have to confirm and characterize (i. e., at the functional level) the molecular defect. The most appropriate therapy could be choosen according to characterization of the molecular defect. For example, for MDR3 deficiency, while 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. For mutation leading to protein truncation, no response to UDCA is expected. In this situation, cell or gene therapy could be proposed. For mutation leading to residual activity, a positive response to UDCA could be expected. In this situation the choice of therapeutic options between the

pursuit of UDCA and the decision to start cell/gene therapy or a combination of UDCA 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 will probably facilitate their application to children with PFIC1-3. In order to be optimal, such diagnostic and therapeutic approaches should be performed in the context of multicenter trials.

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