A 27-month-old girl presented with a short history of jaundice initially attributed to drug-induced liver injury. During the preceding 20 days, she had received a 10-day course of cefprozil and 2 doses of a homeopathic preparation of cantharidin for cystitis. Severe conjugated hyperbilirubinemia was present with normal γ-glutamyl transpeptidase activity. Liver biopsy revealed marked canicular and hepatocellular cholestasis, with moderate hepatocellular disarray, as well as evidence of chronicity, including moderate portal-tract and perisinusoidal fibrosis. Immunohistochemical studies revealed that bile salt export pump expression was preserved, whereas canicular γ-glutamyl transpeptidase expression was largely absent. An inherited cholestatic disorder was suspected. The entire coding region of \( \text{ABCB11} \), encoding bile salt export pump, was analyzed. The patient was found to be a compound heterozygote for the missense mutation c.3148C>T (p.Arg1050Cys) associated with progressive familial intrahepatic cholestasis type 2 in the homozygous state and for the nonsense mutation c.3904G>T (p.Arg1302Ter) associated with progressive familial intrahepatic cholestasis type 2. Despite initial improvement with ursodeoxycholic acid, over the course of 5 years the patient developed cirrhosis that required liver transplant. Our report emphasizes the need for molecular studies even in patients with putatively “explained” cholestasis to reveal the entire spectrum of inherited cholestatic disorders.

Cholestasis may reflect disturbances of bile-acid homeostasis mediated by transport proteins (“transporters”) in hepatocyte and cholangiocyte membranes. Conjugated bile acids are secreted into bile by bile salt export pump (BSEP), a transporter at the hepatocyte canalculus. Variants of the BSEP-encoding gene, \( \text{ABCB11} \), that cause absolute or functional BSEP deficiency confer susceptibility to progressive familial intrahepatic cholestasis (PFIC) type 2 (PFIC2), benign recurrent intrahepatic cholestasis (BRIC) type 2 (BRIC2), and drug-induced cholestasis (DIC).

We describe a girl with severe cholestasis, initially attributed to drug-induced liver injury (DILI), who proved to be a compound heterozygote for PFIC2-associated and BRIC2-associated \( \text{ABCB11} \) mutations. Over 5 years, her status evolved from DILI, DIC, and/or BRIC2 to PFIC2, requiring liver transplant.

**CASE REPORT**

A 27-month-old girl with a 6-day history of jaundice and dark urine, born to nonconsanguineous parents after an unremarkable first pregnancy, was...
referred for care. She had experienced palmar pruritus from infancy. A lower–urinary tract infection with Escherichia coli, diagnosed 20 days earlier, had been treated with oral cefprozil (30 mg/kg per day) for 10 days. Because dysuria persisted, she received 2 doses of oral cantharidin homeopathically compounded (“cantharis,” 200 mg each, 2 days apart). She developed jaundice 5 days after the first dose. Her personal and family history were otherwise unremarkable. Examination revealed icterus, scratch marks, and hepatomegaly. Laboratory investigations revealed a normal hemogram and international normalized ratio (0.85), elevated total bilirubin and direct bilirubin values (19 and 10.13 mg/dL, respectively), elevated aspartate transaminase activity (113 IU/L), and normal alanine transaminase and γ-glutamyl transferase (GGT) activity (46 and 7 IU/L, respectively). Serum copper and ceruloplasmin, urine copper, and serum α-1-antitrypsin values all were normal. No evidence for hepatotropic-virus infection or autoimmunity was found. Abdominal ultrasonography and MRI revealed no abnormality. DILI caused by cefprozil or cantharidin was considered, although published reports were lacking.

Liver biopsy revealed marked canalicular and hepatocellular cholestasis with moderate hepatocellular disarray, portal-tract fibrosis, and perisinusoidal fibrosis (Fig 1 A–C). Portal tracts and lobules were inflamed. Interlobular bile ducts appeared unremarkable; ductular reaction was minimal. Orcein staining disclosed scant metallothionein deposits in perportal hepatocytes. On immunohistochemical study, many hepatocytes marked ectopically for the cholangiocyte-associated antigen cytokeratin 7 (Fig 2A). Like metallothionein deposits, such marking is generally observed in prolonged cholestasis and thus interpreted as indicating subclinical chronicity. BSEP expression was preserved (Fig 2B), whereas canalicular expression of GGT, an ectoenzyme, was nearly absent (Fig 2C). Expression of the transporter multidrug resistance–associated protein 2 was preserved but with displacement to basolateral aspects of hepatocytes (Fig 2D), a nonspecific phenomenon in cholestasis. Another ectoenzyme, alanyl aminopeptidase, and a structurally similar molecule without enzymatic activity, biliary glycoprotein (carcinoembryonic antigen), were well expressed along bile canaliculi (not shown).

The child’s jaundice deepened. On the ninth day of hospitalization, ursodeoxycholic acid (UDCA) was begun, with gradual improvement seen (total bilirubin 8.73 mg/dL and direct bilirubin 6.32 mg/dL after 1 month). Mutation in ATP8B1 or ABCB11 was considered because cholestasis associated with mutation in either is generally not partnered with elevated serum GGT activity. Bland canalicular cholestasis characterizes ATP8B1 disease; this patient, however, had substantial lobular disarray. That BSEP was expressed was consonant with age at presentation with icterus because the absence of BSEP expression characterizes PFIC2, which manifests in infancy. Thus, ABCB11 was clinically and histopathologically the initial candidate for Sanger sequencing (the technique then available). Analysis of its entire coding region revealed compound heterozygosity for 2 known pathogenic mutations: a paternal missense mutation, c.3148C>T (GenBank Single Nucleotide Polymorphism Database identifier rs72549398; Human Gene Mutation Database [HGMD] identification CM042275), which is predicted to cause the non synonymous amino-acid change p.Arg1050Cys, and a maternal nonsense mutation, c.3904G>T (HGMD identifier CM081484), which is predicted to introduce a premature stop codon at p.1302 (p.Glu1302Ter). The paternal mutation has been found in BRIC2. The maternal mutation has been found in PFIC2. Given 2 pathogenic mutations in trans, we did not at that time sequence ATP8B1. Exomic sequencing that included ATP8B1 was, however, undertaken subsequently.

On follow-up, hyperbilirubinemia subsided without resolving. When the parents withdrew UDCA on their own, pruritus reappeared. Three similar exacerbations of cholestasis were recorded approximately once per year. No triggering events were identified.

After 2 years lost to our care, the patient was hospitalized again, 5 years after the original presentation. Jaundice, pruritus, and hepatomegaly were apparent, with conjugated hyperbilirubinemia, elevated transaminase activity and international normalized ratio values, hypoalbuminemia, and pancytopenia ascribed to hypersplenism. MRI results confirmed hepatomegaly, with nodularity and perportal edema, splenomegaly, and gallbladder sludge and stones. Esophageal variceal rupture prompted referral for living-related liver transplant, on which molecular analysis results confirmed the ABCB11 mutations previously identified and revealed heterozygosity for the ABCB11 variant c.1331T>C (p.Val444Ala), which is known to reduce BSEP expression, and the unusual TJP2 (tight junction protein 2) variant c2732A>G (p.Tyr911Cys) (GenBank Single Nucleotide Polymorphism Database identifier rs780609130; HGMD identifier CM154706), which was of uncertain pathogenicity. The explanted liver was cirrhotic without malignancy. At this writing, 2 years after transplant, the child is well.
DISCUSSION

When cholestasis occurs in combination with normal GGT values, to suspect inherited disorders is important. This 27-month-old girl presented with severe cholestasis, originally thought to represent DILI, and persistently low GGT activity. Although DILI associated with alterations in canalicular-transporter expression and function is under active study, correlations among clinical-chemistry, histopathologic, and genetic findings are not generally sought in patients with DILI and/or DIC. In our patient, chronic cholestasis could be ascribed to compound heterozygosity for pathogenic mutations in ABCB11, with drug exposure being a trigger of rather than causing acute intrahepatic cholestasis per se. Thus, events recapitulated the spectrum of BSEP-related liver disease, from DILI manifest as DIC through BRIC2 to PFIC2.

Familial intrahepatic cholestasis includes disorders of reduced bile flow without anatomic obstruction, viz, progressive familial intrahepatic cholestasis (PFIC), BRIC, and

FIGURE 1
Histologic findings: liver-biopsy specimen (all magnifications of original images). A, Moderate hepatocellular disarray with cholestasis, cytoplasmic swelling, and cell plate thickening. An apoptotic hepatocyte is seen (short arrow). Bile-duct profiles (long arrows) are preserved in a portal tract (hematoxylin and eosin; ×200). B, Prominent canalicular cholestasis (arrows; bile plugs) accompanied by hepatocellular swelling and anisocytosis (hematoxylin and eosin; ×400) in this centrilobular region. C, Reticulin stain demonstrating fibrosis of portal tracts, both in isolation (short arrow) and with extension perisinusoidally (long arrow) into the lobule (×40).
intrahepatic cholestasis of pregnancy (ICP). Until recently, PFIC was classified into 3 types; a fourth variant is newly distinguished. These are associated with mutations in ATP8B1 (PFIC1), ABCB11 (PFIC2), ABCB4 (PFIC3), and TJP2 (PFIC4). Mutations in ATP8B1 and ABCB11 can also result in milder phenotypes (BRIC1 and BRIC2, respectively). However, intermediate phenotypes form a continuum between BRIC (mild and transitory) and PFIC (severe and permanent). ICP is BRIC triggered by hormonal shifts in gestation; all 4 genes may contribute. ICP overlaps with DIC in which exogenous hormones are implicated, such as those in contraceptive agents. How paraneoplastic cholestasis (Stauffer syndrome) reflects genetic constitution is poorly understood.

Unlike most cholestatic conditions, PFIC1, BRIC1, PFIC2, BRIC2, and PFIC4 are characterized by normal or near-normal GGT values. GGT activity in PFIC3 is high; intermittently manifest disease is, oddly, not referred to as BRIC3. Within low-GGT familial intrahepatic cholestasis, ABCB11 disease exhibits higher transaminase activity than does ATP8B1 disease, as does TJP2 disease (personal observations).
In our patient, transaminase elevations and histopathologic features suggested ABCB11 rather than ATP8B1 as being likely mutated (severe TJ2 disease had not been defined when she was first evaluated). Compound heterozygosity for 2 known pathogenic ABCB11 mutations was found. Homozygosity for the paternal mutation c.3148C>T underlay BRIC2 in 2 adult sisters.2 Expression of BSEP was not assessed histopathologically, but in vitro assay yielded predominantly immature BSEP.15 The maternal mutation c.3904G>T is recorded in the compound heterozygous state in 3 unrelated patients with PFIC2.3 Among them, 2 were heterozygotes for splice site mutations (c.2178+G>A and c.611+1G>A). Neither expressed BSEP. In the third, who was a heterozygote for the missense mutation c.890A>G (p.Glu297Gly), BSEP expression was scant. That our patient expressed immunohistochemically demonstrable BSEP indicates functional rather than absolute BSEP deficiency, as with combinations of other ABCB11 mutations in patients with intrahepatic cholestasis.15

When our patient received UDCA, clinical and biomarker status improved, but she was not freed from disease: episodes of cholestasis recurred, and cholelithiasis and cirrhotic hepatomegaly developed, with portal hypertension manifested as splenomegaly, hypersplenism, and variceal hemorrhage, requiring liver transplant. She moved from DIC and/or BRIC to PFIC.

Two mechanisms explain failure of GGT values to rise despite conjugated hyperbilirubinemia.16 GGT at the canalicular membrane gains access to plasma when leached by bile salts into bile that seeps between damaged hepatocytes into the space of Disse. If bile lacks bile salts, as in ABCB11 disease, GGT activity in serum remains low. That GGT is normally expressed along bile canaliculi in PFIC2 supports this hypothesis. Alternatively, disordered trafficking of ectoenzymes, such as GGT, to the canalicular membrane (as in microvillus inclusion disease [personal observations] or arthrogryposis–renal-dysfunction–cholestasis syndrome17,18) or abnormal composition of the canalicular membrane (as in ATP8B1 disease19) may abrogate canalicular GGT expression, without which serum GGT activity remains low. We expect that BSEP in our patient was dysfunctional because biomarkers reflected hepatocellular injury, which we ascribe to retention of bile salts within hepatocytes. Heterozygosity for the TJP2 mutation found is, we think, likely noncontributory to cholestasis; it has been reported in the homozygous form in an adult patient with deafness20 but without clinical liver disease (X. Gu, PhD, personal communication, 2018), suggesting limited pathogenicity. We can offer no explanation based on ABCB11 or TJP2 mutation for the deficiency of GGT expression while 2 other canalicular species similar in cell-membrane attachment, biliary glycoprotein, and alanyl aminopeptidase, were unremarkably expressed. PFIC2, with unremarkable canalicular GGT expression, is clinically a steady state. DIC and/or BRIC, by contrast, are in flux during onset and again during resolution. Perhaps transient deficiency of GGT expression is usual in BRIC2; the point has not been studied. GGT handling in the cholestatic liver clearly awaits investigation.

CONCLUSIONS

Inherited disorders should be considered in cholestasis with normal GGT activity even when the clinical history suggests DILI and/or DIC. Molecular diagnostics can reveal novel combinations of mutations associated with cholestatic disorders, as in our patient. Case descriptions will help to elucidate the full spectrum of inherited cholestasis and permit terminology that meaningfully addresses genetic defects and clinicopathologic features.

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ABBREVIATIONS

BRIC: benign recurrent intrahepatic cholestasis
BRIC1: benign recurrent intrahepatic cholestasis type 1
BRIC2: benign recurrent intrahepatic cholestasis type 2
BSEP: bile salt export pump
DIC: drug-induced cholestasis
DILI: drug-induced liver injury
GGT: γ-glutamyl transferase
HGMD: Human Gene Mutation Database
ICP: intrahepatic cholestasis of pregnancy
PFIC: progressive familial intrahepatic cholestasis
PFIC1: progressive familial intrahepatic cholestasis type 1
PFIC2: progressive familial intrahepatic cholestasis type 2
PFIC3: progressive familial intrahepatic cholestasis type 3
PFIC4: progressive familial intrahepatic cholestasis type 4
TJP2: tight junction protein 2
UDCA: ursodeoxycholic acid
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