MYO5B Gene Mutations: A Not Negligible Cause of Intrahepatic Cholestasis of Infancy With Normal Gamma-Glutamyl Transferase Phenotype

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ABSTRACT

Objectives: Progressive familial intrahepatic cholestasis is an expanding group of autosomal recessive intrahepatic cholestatic disorders. Recently, next-generation sequencing allowed identifying new genes responsible for new specific disorders. Two biochemical phenotypes have been identified according to gamma-glutamyltransferase (GGT) activity. Mutations of the myosin 5B gene *(MYO5B)* are known to cause microvillus inclusion disease. Recently, different mutations in *MYO5B* gene have been reported in patients with low-GGT cholestasis.

Methods: A multicenter retrospective and prospective study was conducted in 32 children with cryptogenic intrahepatic cholestasis. Clinical, biochemical, histological, and treatment data were analyzed in these patients. DNA from peripheral blood was extracted, and all patients were studied by whole exome sequencing followed by Sanger sequencing.

Results: Six patients out of 32 had mutations in the *MYO5B* gene. Of these six patients, the median age at disease onset was 0.8 years, and the median length of follow-up was 4.2 years. The most common signs were pruritus, poor growth, hepatomegaly, jaundice, and hypocholic stools. Two patients also showed intestinal involvement. Transaminases and conjugated bilirubin were moderately increased, serum bile acids elevated, and GGT persistently normal. At anti-Myo5B immunostaining, performed in liver biopsy of two patients, coarse granules were evident within the cytoplasm of hepatocytes while bile salt export pump was normally expressed at the canalicular membrane. Six variants in homozygosity or compound heterozygosity in the *MYO5B* gene were identified, and three of them have never been described before. All nucleotide alterations were located on the myosin motor domain except one missense variant found in the isoleucine-glutamine calmodulin-binding motif.

Conclusions: We identified causative mutations in *MYO5B* in 18.7% of a selected cohort of patients with intrahepatic cholestasis confirming a relevant role for the *MYO5B* gene in low-GGT cholestasis.

Key Words: cholestasis, mutations of the myosin 5B gene, next generation sequencig, progressive familial intrahepatic cholestasis

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What Is Known

- Myosin 5B gene *(MYO5B)* mutations are associated with microvillus inclusion disease (MVID). Different mutations in the same gene have been recently described in patients with normal gamma-glutamyl-transferase (GGT) cholestasis.
- Severe biallelic mutations are less frequent in isolated cholestasis than in MVID patients.

What Is New

- Mutations in MYO5B can cause >15% of intrahepatic cryptogenic cholestasis which exhibits the characteristics of low GGT cholestasis.
- Mutations in MYO5B may induce cholestasis due to an altered BSEP localization on the canalicular membrane. However, at least in some patients, BSEP is preserved and correctly localized.
- Some MYO5B mutations (p.Q252X, p.P517L, p.R92C, and p.I192Sfs∗47) located on the myosin head motor domain are correlated with an altered Myo5B expression but not with expression and/or localization of BSEP.

Progressive familial intrahepatic cholestasis (PFIC) (1), is a heterogeneous group of cholestatic liver disorders underlying different pathogenetic mechanisms and, in most cases, transmitted with autosomal recessive inheritance. Several phenotypes of PFIC originate from a disturbed secretion of bile from hepatocytes resulting in hepatocellular cholestasis, which usually occurs in the first year of life. PFIC was firstly distinguished based on serum

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gamma-glutamyltransferase (GGT) activity in two distinct phenotypes, normal (GGT < 100IU/L) and high GGT PFIC (2). Subsequently, three distinct types of PFIC were described, caused by the dysfunction of specific proteins essential for bile salts secretion and identified as type 1 and type 2 PFIC presenting with a persistently normal/low GGT, and type 3 with high GGT (3,4). Mutations in the genes *ATP8B1* (FIC1) (5) and *ABCB11* [bile salt export pump (BSEP)] (6,7) generated PFIC 1 and 2 while those in *ABCB4* (mDR3) (8) resulted in PFIC 3.

Most patients that are compound heterozygous or homozygous for mutations in the gene *ABCB11* coding for BSEP, the primary bile salt export pump expressed on the hepatocyte canalicular membrane, have normal GGT; however, to date, up to one third of children with low GGT cholestasis phenotype did not have mutations in the ATB8B1 or ABCB11 genes (9,10). In recent years new genes, such as *MYO5B,* have been associated with PFICs after the detection of mutations in some patients (11–16).

Biallelic mutations in *MYO5B* were first associated with microvillus inclusion disease (MVID), a congenital disorder of enterocytes leading to intractable diarrhea, and subsequently described in patients with normal GGT cholestasis (11–16).

Myosin 5B is essential for plasma membrane recycling and epithelial cell polarization interacting with RAB11A and allowing the normal trafficking of ABC transporter proteins, including BSEP, to the canalicular membrane (17–20). Some authors (11,12,21) reported that mutations in the *MYO5B* gene might cause incorrect BSEP localization, thus altering bile salt secretion and eventually causing cholestasis, although recent data do not seem to support this hypothesis (13,14). Overeem et al (22), have recently elucidated the pathogenetic mechanism of these variants in the Myosin5b motor domain by demonstrating that specific missense mutations lead to incorrect localization of the canalicular protein in hepatocytes by inducing toxic gain-of-function via RAB11A.

The present study analyzed six patients with intrahepatic cholestasis of unknown origin, belonging to a larger cohort of 32 patients recruited in the last 4 years, in which we found mutations in MYO5B. In the rest of the cohort patients, we found mutations in genes already known to cause cholestasis.

METHODS

Patients

A multicenter, retrospective, and prospective study on children with intrahepatic cholestasis was conducted. Patients, together with their parents, were enrolled from 2016 to 2020 according to the following inclusion criteria such as the age at disease onset < 18 years, and at least one of these characteristics: direct bilirubin > 1.5 mg/dL, serum GGT >200U/L, total serum bile acid (TSBA) >50 μmol/L, signs of cholestasis at liver biopsy and absence of other causes of liver disease. Retrospective patients included 24 children with undiagnosed cholestasis who had previously undergone genetic analysis without finding mutations in the *ATP8B1, ABCB11,* and *ABCB4* genes. Meanwhile, eight patients diagnosed with cholestasis who fulfilled the inclusion criteria were prospectively enrolled. The Ethical Committee approved the study and patients' parents signed written informed consent. Clinical features, biochemical, molecular, and histological data were collected and analyzed.

Whole Exome Sequencing

DNA from peripheral blood was extracted using the QIAsymphony workstation and the QIAsymphony DNA kits (Qiagen). DNA quantity and quality were assessed with Qubit (Thermo Fisher Scientific) and agarose gel. For each patient, 1 ug of genomic DNA was sent to Macrogen Inc company (Korea, Seoul) for whole exome sequencing using Illumina Platform. The Human All Exon V6 Library kit (Agilent) was employed to select the target regions (60.5 Mb), whereas the NovaSeq 150pb PE sequencing kit was chosen for sequencing at an average coverage of 100×.

Histology and Immunohistochemical Analysis

Liver tissue was formalin-fixed and paraffin-embedded. Hematoxylin-eosin (HE), periodic acid-Schiff (PAS), PAS after diastasis, and trichrome of Masson were routinely analyzed. Immunohistochemistry was made with anti-cytokeratin7 (CK7) (monoclonal mouse anti-human, OV-TL12/30, ready to use; Agilent, Santa Clara, CA, USA), anti-CK19 (monoclonal mouse anti-human, RCK108, ready to use; Agilent), anti-MYO5B (rabbit polyclonal, C-term human MYO5B, LS-B3118/122815, Life Span BioSciences, Inc, Seattle, WA, USA, 1:100) and anti-BSEP (rabbit polyclonal, NBP1-89319, Novus Biologicals, Centennial, CO, USA, 1:1000). Normal liver (donor's livers at time 0) was used as a positive control.

Bioinformatics Analysis and Ultrastructural analysis: see supplemental content, *http://links.lww.com/MPG/C679*.

RESULTS

Patient's Clinical Characteristics

We identified 6 of 32 (18.7%) patients having mutations in the *MYO5B* gene, respectively 2 of 8 (25%) in the prospective cohort, and 4 of 24 (17%) in the retrospective cohort. At disease onset and last evaluations, the median age was 0.8years (0.1–1.5years) and 5.2 years (2.1–16.4 years). The median length of follow-up was 4.2 years (0.8–16.3 years). The patient's characteristics are reported in Table 1. Genetic analysis is documented in Table 1, Supplemental Digital Content, *http://links.lww.com/MPG/C676*.

Patient 1

This 15-month-old Caucasian boy, born at full term to unrelated parents, had a negative family history and suffered from jaundice and severe itching upon hospital admission. Blood exams revealed a moderate increase of transaminases, conjugated hyperbilirubinemia, elevated TSBA, normal GGT, and prothrombin time. The abdominal ultrasound was normal. Ursodeoxycholic acid (UDCA), rifampicin, and fat-soluble vitamins (FSV) were started. The patient suffered from fluctuating pruritus during follow-up, and antihistamine medication was added. At his last visit, he was 4.8 years of age and presented intermittent pruritus. Transaminases were 1.5 × upper limit of normal (ULN), with normal bilirubin, GGT, and only a mild increase of TSBA (22 μmol/L). He never had chronic diarrhea. He was compound heterozygous for the known (rs777038090) damaging missense variant c.C2470T; p.R824C, located on the highly conserved isoleucine-glutamine (IQ) calmodulin-binding motif, and for the recently reported missense variation c.A1961G; p.Y654C predicted as damaging and located in the head, motor domain. Sanger results confirmed both mutations on proband and the state of carriers on parents, the father of the R824C missense mutation and the mother of the Y654C missense mutation (Fig. 1.A, Supplemental Digital Content, *http://links.lww.com/MPG/C678*).

Patient 2

This Caucasian boy was born at term from unrelated parents. Medical family history was unremarkable. He had severe diarrhea

TABLE 1. Patient's characteristics at disease onset and outcome

Patient	Sex	Country	Age at onset (y)	Age last visit (y)	Alt (nv <40 U/L)	GGT (U/L)	BT/BD (mg/dL)	Bile acids (nv 2–10 μmol/L)	Clinical features	Intestinal disease	Treatment	Outcome
1	M	Bosnia	1.3	4.8	64	13	6.2/5.2	496	Jaundice, pruritus	No	UDCA, FSV, rifampicin, antihistamine	Fluctuation of pruritus
2	M	Italy	0.3	5.3	199	20	2/1.2	68	Poor growth, pruritus, diarrhea, hepatomegaly	Yes	UDCA, FSV, rifampicin, antihistamine, naloxone, cholestyramine, prednisone	Intestinal disease and cholestasis improvement
3*	F	Morocco	1.7	5.1	213	12	2.2/1.2	476	Poor growth, pruritus, hepatomegaly, hypocholic stools		UDCA, FSV, rifampicin	Fluctuation of pruritus
4*	M	Morocco	1.3	2.1	54	45	0.4/0.1	19	Pruritus	No	UDCA, FSV, rifampicin	Fluctuation of pruritus
5	M	Italy	0.2	16.4	–	–	v	–	Poor growth, diarrhea, pruritus, hepatomegaly	Yes	UDCA, FSV, rifampicin, cholestyramine	Pruritus well-controlled, intermittent diarrhea
6	F	Morocco	0.2	10.8	357	24	5.1/4.9	28	Jaundice, pruritus, hepatomegaly	No	UDCA, FSV, rifampicin, antihistamine	Pruritus well-controlled at last follow-up

ALT = alanine aminotranferase, FSV = fat-soluble vitamin; nv = normal value; UDCA = ursodeoxycholic acid. *Siblings.

with abdominal distension and poor growth since the first months of his life. For this reason, he was hospitalized at the age of three months. During hospitalization, severe pruritus and hepatomegaly were observed. An increase in transaminase activity and conjugated bilirubin was found with normal GGT and prolonged prothrombin time (PT INR 2.20) normalizing after parenteral vitamin K. TSBA were elevated (68.2–161 μmol/L). Abdominal ultrasound confirmed hepatomegaly with a normal biliary tree. UDCA, rifampicin, FSV, and sodium bicarbonate for metabolic acidosis were started, and an intestinal biopsy was performed.

Only mild alterations were evident on histology: mildly blunted villi and slightly increased inflammatory cells within lamina propria (Fig. 1A–C). Under transmission electron microscopy, few intracytoplasmic microvillus inclusions were recognizable in the apical surface of epithelial cells (Fig. 2). The microvillus inclusions were complete brush borders with a microvillus membrane, microfilaments, and a surface filamentous coat. Epithelial cells showed regularly organized microvilli with a visible terminal web; sometimes, they appeared dilated as pseudopodial evaginations. These features were consistent with MVID. During follow-up, he presented bouts of MVID-associated cholestasis with severe pruritus, also treated with cholestyramine, antihistamine, and naltrexone. A liver biopsy displayed preserved architecture in the absence of inflammation and cholestasis. CK7 immunostaining

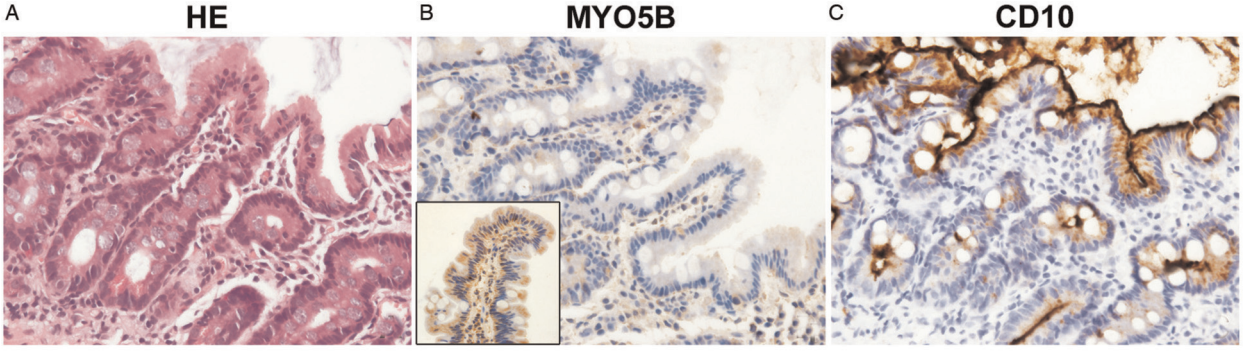
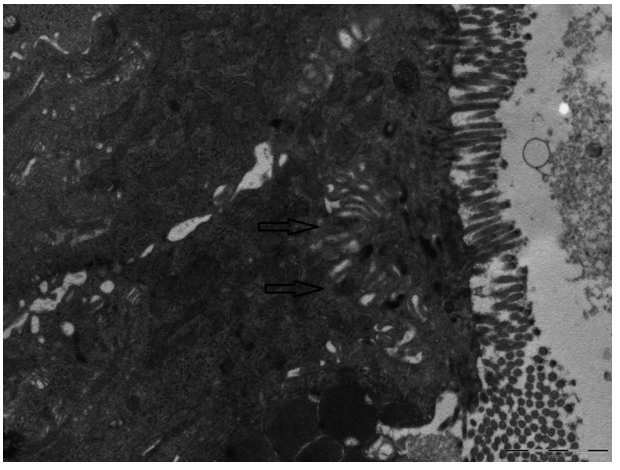


FIGURE 1. Duodenal biopsy and electronic microscopy of patient 2. Duodenal biopsy (FIGURE 1 1demonstrated mildly blunted villi (HE, 20×). The loss of the functional myosin 5B protein results in reduced/absent Myo5B expression at brush border surface (inset: immunostaining in normal control) (20×). Preserved apical CD10^{b} immunostaining at brush border surface in duodenal villus enterocytes (20×) is observed. At electronic microscopy (Fig. 2), the intestinal epithelial cell shows thinned out microvilli with quite regular morphology. A microvillus inclusion, located close to the apical surface (arrow), is easily detectable. HE = hematoxylin-eosin.



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showed a ductular reaction at the periphery of the portal tracts. Neither sign of acute cholangitis nor periductal as well as lobular fibrosis was evident (Fig. 3A). BSEP was normally expressed on immunohistochemistry at the canalicular membrane (Fig. 3B). Anti-Myo5B showed coarser granules within the cytoplasm of hepatocytes (Fig. 3C and D) more significant than in the normal liver.

Severe diarrhea with metabolic acidosis persisted, transiently requiring total parenteral nutrition. A treatment attempt with corticosteroids was performed, leading to diarrhea improvement. He had average growth at 5 years of age at the last follow-up, without pruritus and diarrhea. Blood exams revealed normal bilirubin, transaminases, and TSBA. Exome sequencing analysis led to the identification of two new variants, located on the myosin motor-head: c.C1550T; p.P517L and c.C754T; p.Q252X (rs774139620), which introduces a premature stop codon. By Sanger sequencing, both variants were confirmed in trans in the patient, and both parents carried a single child's mutation in heterozygosis. The mother carries the stop codon and the father the missense variant (Fig. 1B, Supplemental Digital Content, *http://links.lww.com/MPG/C678*).

Patients 3 and 4

Patient 3 was a Moroccan girl, born at term from unrelated parents, presented at 19 months of age for severe pruritus, hypocholic stools, poor growth, and mild hepatomegaly. Blood exams showed raised transaminase levels, conjugated hyperbilirubinemia, normal GGT, and markedly increased TSBA. The abdominal ultrasound was normal. UDCA, rifampicin and FSV were started. During the following 3.5 years, she presented fluctuation of pruritus responded to medical treatment and normalization of transaminase activity. She never presented chronic diarrhea. Patient 4 was the sibling of patient 3. Cholestasis was detected at 15 months of age due to family screening. Clinical examination was regular for age, and he was asymptomatic. Indeed, at blood exams, he had a mild increase in ALT value and moderate elevation of TSBA with normal bilirubin and GGT. The abdominal ultrasound was normal. UDCA and FSV were started. During the following months, he suffered from pruritus and bile acids increased to the value of 303 μmol/L. Rifampicin was added with pruritus improvement. Like his sister, he never presented chronic diarrhea. Genetic analysis identified homozygosity for the missense damaging variant c. C2470T; p.R824C confirmed in both siblings and the mother. Father's DNA was not available (Fig. 1C, Supplemental Digital Content, *http://links.lww.com/MPG/C678*).

Patient 5

Patient 5 was a Caucasian boy, born at term, with a negative familial history. He presented at two months of age with diarrhea and poor growth. At 1 year of age, severe pruritus and hepatomegaly were observed. Blood exams showed an increase of transaminases (ALT2 × ULN), conjugated hyperbilirubinemia, normal GGT, and prolonged prothrombin time normalized after parenteral vitamin K administration. Abdominal ultrasound revealed a normal biliary tree. UDCA, FSV, and rifampicin were started. Over the next few years, he experienced fluctuation in pruritus and several episodes of acute enteritis with electrolyte and metabolic imbalance that required hospitalization but ultimately self-limited. esophagogastroduodenoscopy proved normal histology, and electronic microscopy was not performed. Cholestyramine was added to medical treatment. He underwent two liver biopsies, the first at disease onset and the second during follow-up. Both indicated conserved architecture without inflammation, cholestasis, and fibrosis (Fig. 3E). On immunohistochemistry, BSEP was normally present at the canalicular pole of the hepatocytes (Fig. 3F). Anti-Myo5B showed coarser granules within the cytoplasm of hepatocytes (Fig. 3G and H) more considerable than in the normal liver.

When he was 16 years old at the last clinical evaluation, he presented mild hepatomegaly and normal liver function. Pruritus was well-controlled by rifampicin. He resulted in a compound heterozygote for the protein-truncating deletion variant c.574delA; p. I192Sfs∗47 and for the known (rs372682296) missense variant c. C274T; p.R92C, both located on the myosin motor domain. Both variants were confirmed in trans in the patient and present in the parents (Fig. 1D, Supplemental Digital Content, *http://links.lww.com/MPG/C678*).

Patient 6

The patient was a Moroccan girl who presented jaundice at two months of age and severe pruritus since her four months. She was born at term from unrelated parents. Family medical history was characterized by pregnancy's cholestasis in the mother. Clinical examination showed hepatomegaly, and the blood exams revealed elevated transaminase levels, conjugated hyperbilirubinemia, normal GGT, and increased TSBA (up to 223 μmol/L). UDCA, rifampicin, and FSV were started, achieving the normalization of transaminases and bilirubin. During follow-up, she presented persistent cholestasis and pruritus until 9 years. Cetirizine therapy was added. At last follow-up, at 10 years of age, she was in good clinical condition, without jaundice or pruritus, normalized blood exams, and an average value of hepatic elastometry (5.9 kPa). Whole exome sequencing (WES) analysis identified a homozygous missense pathogenic mutation c.C2470T; p.R824C situated on the IQ calmodulin-binding motif on the motor domain. The mutations were confirmed by Sanger sequencing, and the parents carried a single copy of the missense mutation present on the child. (Fig. 1E, Supplemental Digital Content, *http://links.lww.com/MPG/C678*).

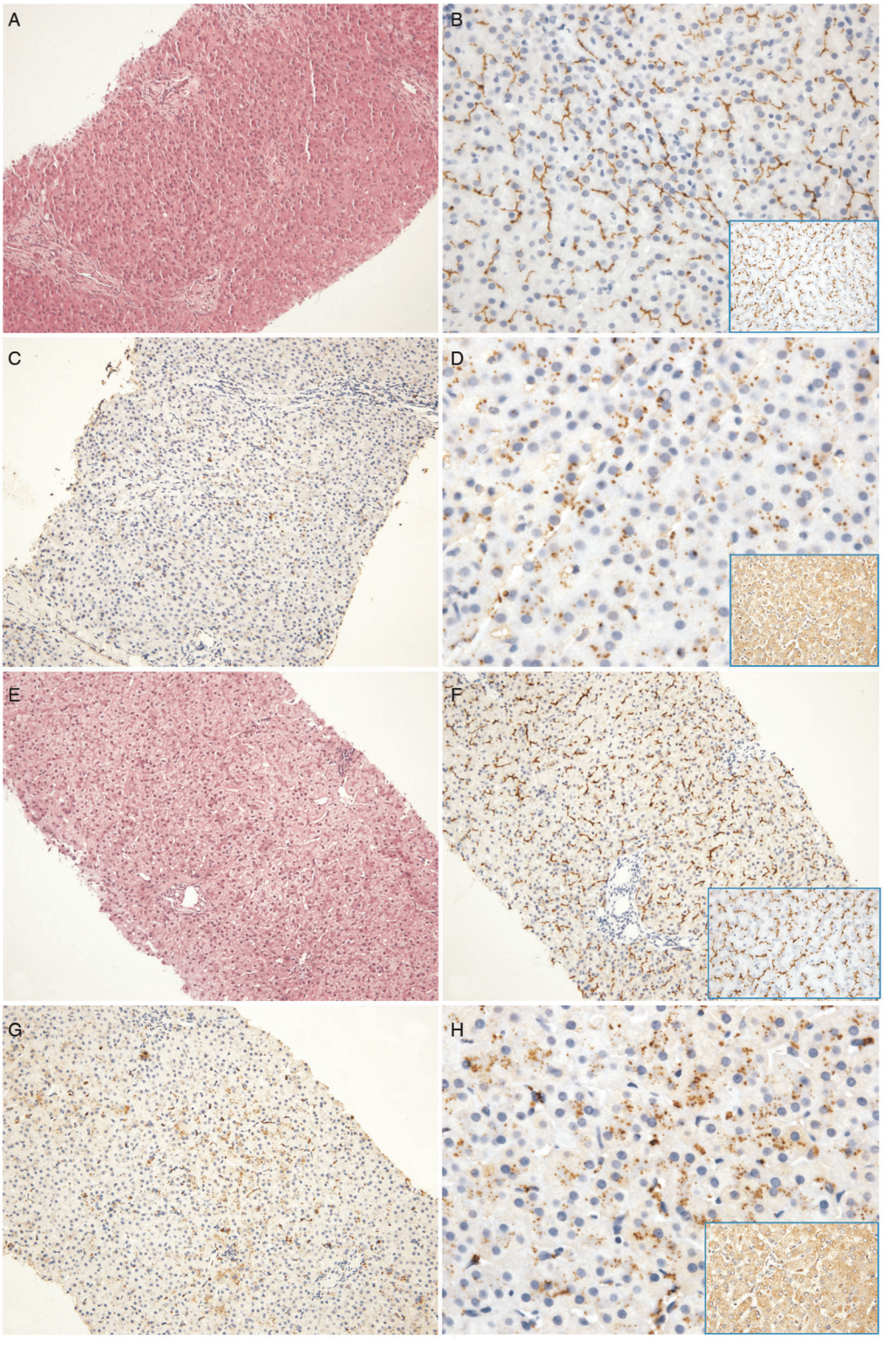

FIGURE 3. Liver biopsies of cases 2 (A–D) and 5 (E–H). Liver biopsies demonstrated in both cases 2 and 5 only minimal changes: mild edema of the portal tracts with minimal fibrosis and inflammation (A–E, HE 20×). Immunostaining demonstrated a preserved BSEP expression at the canalicular pole (B–F, 40×,20×; inset normal control, 40×). Expression of MYO5B manifested by coarse cytoplasmic granules in both casas (C–G, 20×.D–H,40×) but fine granules in control (inset) (40×). HE = hematoxylin–eosin; MYO5B = mutations of the myosin 5B gene.

DISCUSSION

PFICs 1 and 2 are characterized by the failure of bile acids secretion with an accumulation of bile products in the liver causing progressive parenchymal damage. In the recent past, the diagnosis was based mainly on clinical, biochemical, and histological data and the subsequent finding of mutations in specific genes. Thanks to next generation sequencig (NGS) techniques, molecular confirmations in clinical diagnoses have become increasingly widespread in recent years. To date, the most common approach for the identification of pathogenetic mutations is based on panels of targeted genes possibly followed, in case of negativity, by a WES analysis (23). Based on these considerations, more centers in the last years reported their experience with NGS in pediatric cholestasis (24–28), expanding the etiology of PFIC and allowing the identification of new genes, especially in patients with the normal-low GGT PFIC phenotype. In a minority of patients, even with this approach, no mutations have been found, probably because they were not detectable with these techniques or present in genes not yet associated with PFIC.

Particularly interesting seemed the role of *MYO5B,* a gene involved in regulating membrane trafficking in polarized epithelial cells such as hepatocytes, enterocytes, and respiratory epithelial cells. Mutations in the *MYO5B* gene were known to cause MVID (OMIM 251850), an autosomal-recessive congenital disorder of intestinal epithelial cells, causing severe diarrhea with neonatal-onset, usually requiring total parenteral nutrition and even intestinal transplantation (29–32).

In the liver, RAB11A regulated the normal trafficking of ABC transport proteins, such as BSEP, from the trans-Golgi network via positive apical recycling endosomes to the hepatocyte canalicular membrane, playing a key role in BSEP localization and normal canalicular biliary secretion. Mutants in the MYO5 motor domain appeared to inhibit the formation of these specialized endosomes through its interaction with active RAB11A (22).

Cholestasis has been first reported in some MVID patients before or after intestinal transplantation, even unrelated to parenteral nutrition (21,33). Later on, mutations in the *MYO5B* gene were identified, using a WES approach, in 26 patients with normal GGT cholestasis (Table 2, Supplemental Digital Content, *http://links.lww.com/MPG/C677*) (11–16). These subjects had a normal GGT PFIC phenotype with onset in the first two years of life, but also later, characterized by jaundice, severe pruritus, hepatomegaly, conjugated hyperbilirubinemia, raised serum bile salts, persistently low serum GGT activity, and mild to moderately elevated transaminases. During disease, cholestasis could be intermittent, recurrent, or persistent. One patient developed portal hypertension and hypersplenism (16) and two died respectively for a Gram-negative sepsis in early cirrhosis and the second while waiting liver transplantation (12,16).

We reported our experience with 32 cholestatic patients analyzed by a WES approach. We found *MYO5B* mutations in six (18.7%) of them, a similar percentage of those documented by Qiu et al (12) Our patients presented similar characteristics to those previously described, with normal GGT PFIC-like phenotype, a disease onset in the first 2 years of life, and mild cholestasis.

One patient among those described by Gonzales et al (11) presented acute diarrhea before the age of 3 years indeed with normal duodenal histology. Cockar et al (13) observed two subjects with severe intestinal involvement of early-onset, resolved during follow-up. One of our patients also presented severe diarrhea at disease onset, and a diagnosis of MVID was made. Corticosteroids were described in a few patients affected by MVID with no beneficial effects (34); however, in our patient, diarrhea improved after the assumption of corticosteroids and recurred at prednisone withdrawal. During follow-up, when the patient received corticosteroids, also cholestasis improved. We do not know if cholestasis has improved due to corticosteroids therapy, even if we can hypothesize a steroid action similar to that previously described in PFIC2 patients (35).

By immunohistochemical analysis from the liver biopsies of MY05B patients, some authors (11,12,21) observed an abnormal canalicular distribution of BSEP and MDR3. MY05B and RAB11A immunoreactivity were also abnormal, with intense and granular staining in the cytoplasm of hepatocytes but not in the canalicular membrane. Based on these findings, it was supposed that mutations on MY05B could induce cholestasis due to an altered BSEP localization on the canalicular membrane, thus interfering with bile acids secretion; however, was recently observed that in some patients with mutations in MY05B, BSEP was preserved and correctly localized in the canalicular membrane (13,14). In two patients of our cohort, correct BSEP expression and localization were observed, reinforcing the statement that, at least in some patients with MY05B-associated cholestasis, the role of BSEP has yet to be clarified.

In the 26 patients previously reported, different types of treatment were used. Although some patients received only medical treatment, others required surgical intervention and one patient underwent liver transplantation for persistent pruritus and low quality-life (13). At this time, none of our patients required biliary diversion or liver transplantation. Even if ASBT inhibitors are in clinical development for multiple diseases, including cholestatic disorders, none of our patients, like the others previously reported, received this treatment.

Van IJzendoorn et al (36) calculated the prevalence of MVID-associated cholestatic liver disease (CLD) to be 37%. When considered only patients with MVID and *MYO5B* mutations the prevalence was 54%. They suggested that CLD in patients without MVID appears to be a manifestation of mild *MYO5B* mutations, as previously reported by Qiu et al (12), who found severe biallelic mutations less frequently in isolated cholestasis than in MVID. Overeem et al (22) explained the genotype-phenotype correlation in patients with cholestasis without MVID, reporting that only *MYO5B* biallelic mutations affecting the motor domain were associated with the disease and that they were of gain-of-function type. Aldrian et al (37) reported 114 patients with intestinal, cholestatic, or both diseases concluding that loss-of-function mutations cause MVID phenotype altering enterocyte polarization while missense mutations lead to misfolding and MY05B degradation, resulting in lack of MY05B protein.

From the genetic point of view, three of our patients were compound heterozygous, while the other three were homozygous. Three missense variants, R824C, R92C, and Y654C, have already been reported in the literature, but we have identified three other variants never described before. The new variants included a missense variant, one nonsense, and a frameshift mutation that caused the introduction of a premature stop codon. All variants except the missense R824C located on the IQ calmodulin-binding motif were located on the myosin motor domain. No homozygous nonsense or frameshift mutations have been observed in PFIC6 patients, including those reported herein.

In conclusion, MY05B deficiency can cause low GGT cholestasis that closely resembles PFIC 1 and 2 with some differences such as less severe jaundice, lower level of transaminases, later disease onset, and the possible occurrence of diarrhea. Most of the patients reported had mild cholestasis, managed with medical treatment even if cholestasis may be persistent or recurrent during follow-up. Our results strongly supported the role of the MY05B gene and its high prevalence in low-GGT cholestasis, suggesting searching MY05B mutations in all children with normal/low-GGT PFIC, especially

when other causes were excluded. Finally, future studies should investigate mechanisms other than BSEP mislocation.

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