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ORIGINAL ARTICLE

Heterozygous mutations of ATP8B1, ABCB11 and ABCB4 cause mild forms of Progressive Familial Intrahepatic Cholestasis in a pediatric cohort

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KEYWORDS
ATP8B1;
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Abstract
Introduction: Heterozygous defects in genes implicated in Progressive Familial Intrahepatic Cholestasis have been described in milder forms of cholestatic diseases. Our aim is to describe clinical, laboratory and imaging characteristics as well as treatment and outcome of a cohort of pediatric patients with heterozygous mutations in ATP8B1, ABCB11 or ABCB4.
Patients and methods: We present a retrospective descriptive study including pediatric patients with at least one heterozygosis defect in ATP8B1, ABCB11 or ABCB4 diagnosed after a cholestatic episode. Clinical, diagnostic and outcome data were collected including gene analysis (panel of PFIC NextGeneDx®).
Results: 7 patients showed a heterozygous mutation: 3 patients in ABCB4, 1 in ABCB11, 2 in ABCB4 and ABCB11 and 1 in ATP8B1. The median onset age was 5.5 years with a median time of follow-up of 6 years. The initial presentation was pruritus followed by asymptomatic hypertransaminasemia and persistent cholestasis. Two patients had family history of gallbladder stones and mild hepatitis. All showed elevated transaminases and bile acids, high gamma glutamyl-transferase (GGT) in 3 and conjugated bilirubin in 2 patients. Liver biopsy showed inflammatory infiltrate or mild fibrosis with normal immunohistochemistry. All patients were treated with ursodeoxycholic acid, two patients requiring the addition of resincholestyramine. During follow-up, 3 patients suffered limited relapses of pruritus. No disease progression was observed.

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Conclusion: Heterozygous mutations in genes coding proteins of the hepatocellular transport system can cause cholestatic diseases with great phenotypic variability. The presence of repeated episodes of hypertransaminasemia or cholestasis after a trigger should force us to rule out the presence of these heterozygous mutations in genes involved in CIFP.

PALABRAS CLAVE
ATP8B1;
ABCB11;
ABCB4;
BSEP;
MDR3;
FIC1;
Colestasis intrahepática progresiva familiar

Mutaciones en heterocigosis en ATP8B1, ACB11 y ABCB4 como causa de formas leves de Colestasis Intrahepática Progresiva Familiar en una cohorte pediátrica

Resumen
Introducción: Se han descrito defectos en los genes implicados en las colestasis intrahepáticas familiares progresivas (CIFP) causantes de colestasis más leves. El objetivo es estudiar las manifestaciones clínicas, analíticas y de imagen así como la evolución y respuesta al tratamiento de los portadores en heterocigosis de mutaciones en ATP8B1, ABCB11 y ABCB4.
Pacientes y métodos: Estudio descriptivo retrospectivo de pacientes con al menos una mutación en heterocigosis en los genes ATP8B1, ABCB11 o ABCB4 diagnosticados tras un episodio de colestasis. Se recogieron variables demográficas y datos clínicos, diagnósticos, incluyendo estudio genético (panel de CIFP *NextGeneDx*®), tratamiento y evolución.
Resultados: 7 pacientes presentaron al menos una mutación en heterocigosis: 3 en ABCB4, 1 en ABCB11, 2 en ABCB4 y ABCB11 y 1 en ATP8B1. La edad media de inicio fue de 5.5 años con un tiempo medio de evolución de 6 años. La clínica inicial fue prurito seguida de hipertransaminasemia asintomática y colestasis persistente. Dos pacientes tenían antecedentes familiares de litiasis biliar y hepatitis leve. Todos mostraron transaminasas y ácidos biliares elevados, 3 gamma-glutamiltransferasa (GGT) y 2 bilirrubina directa. La biopsia hepática mostró infiltrado inflamatorio o fibrosis leve, inmunohistoquímica normal. Fueron tratados con ácido ursodeoxicólico añadiéndose colestiramina en 2. Durante el seguimiento 3 presentaron episodios autolimitados de prurito. No se observó progresión de la enfermedad.
Conclusiones: Mutaciones en heterocigosis en los genes implicados en el sistema de transporte hepatocelular pueden ocasionar cuadros de colestasis con gran variabilidad fenotípica. Episodios repetidos de hipertransaminasemia o colestasis tras un desencadenante deben hacernos sospechar mutaciones en los genes implicados en las CIFP.

Introduction

Progressive Familial Intrahepatic Cholestasis (PFIC) is a heterogeneous group of recessive autosomal inherited liver disorders that affects genes encoding hepatocellular transport system proteins.[1] This defect results in the inability to suitable form and excrete bile from hepatocytes leading to intrahepatic cholestasis, pruritus, and jaundice evolving to cirrhosis and end stage liver disease.[1–3] Three genes have been classically associated with these diseases (ATPB8B1, ABCB11 and ABCB4) although in recent years other involved genes have been described. Gene ATPB8B1 (18q21-22) encodes the protein FIC1, an aminophospholipid translocase located in the canalicular membrane of hepatocytes and responsible for transporting phospholipids (phosphatidylserinne and phosphaticylethanolamine) inside the canalicular membrane.[1,3] ABCB11 (2q24) codifies Bile Salt Export Pump (BSEP), an ATP-dependent transporter only expressed in the liver, that exports bile acids from inside the hepatocyte to the canaliculi against gradient.[1,2] Multi-Drug Resistance 3 glycoprotein (MDR3), encoded by the gene ABCB4 (7q21), is a floppase that excretes phospholipids (phosphatidylcholina) that neutralizes the detergent effect of hydrophobic bile salts.

Partial defects with heterozygous mutations of these genes have been described in milder forms of cholestatic liver diseases[3,4]: Benign Recurrent Intrahepatic Cholestasis (BRIC), Intrahepatic Cholestasis of Pregnancy (ICP), Low Phospholipid-Associated Cholelithiasis Syndrome (LPAC), Drug Induced Cholestasis (DIC), Drug Induced Liver Injury (DILI), transient neonatal cholestasis or adult biliary fibrosis or cirrhosis[2,5,6] (Table 1). Historically, these have been considered to be milder and benign recessive autosomal hereditary forms, usually not progressive, presenting with limited episodes of cholestasis, pruritus and jaundice, and triggered by a known insult in most of the cases. However, some case reports show evolution to cirrhosis and end-stage liver disease, hence they might be taken as a clinical continuum.[7,8] Little is known about these entities in which the genetic defect affects only partially the protein function, since in the literature there are only clinical cases or short series with a wide spectrum of morbidity. We present

Table 1 Pathologies associated to deffects in ATP8B1, ABCB11 and ABCB4.

ATP8B1	• Progressive familial intrahepatic cholestasis (PFIC) • Benign recurrent intrahepatic cholestasis (BRIC) • Intrahepatic cholestasis of pregnancy (ICP) (low frequency)
ABCB11	• PFIC 2 • BRIC 2 • ICP 2 (low frequency)
ABCB4	• PFIC 3 • BRIC 3[1] • Adult biliary cirrhosis • Cholesterol gallstone disease - Low phospholipid associated cholelitiasis (L-PAC) • Transient neonatal cholestasis • Drug induced cholestasis • ICP 3

Refs. 10, 13, 25.

a series of 7 pediatric patients diagnosed with mild atypical forms of PFIC in order to expand the knowledge.

Patients and methods

Patients

This is a retrospective descriptive study (September 2017 to December 2018), including 7 children with heterozygous gene defects encoding proteins of the hepatocellular transport system, diagnosed after a cholestatic episode defined as high conjugated bilirubin > 1 mg/dl or GGT above normal levels for age. In all of them, other etiologies (infections, autoimmune, alpha-1-antitrypsin deficiency, toxics and Wilson's disease) were excluded. Data referring to familial history, onset symptomatology, laboratory and imaging findings (ultrasound, magnetic resonance cholangiopancreatography), histology, immunohistochemistry, gene analysis, treatment and relapses were collected. Parental informed consent was obtained in all the patients.

Mutation analysis

Genomic DNA was obtained and amplification by polymerase chain reaction (PCR) of exons and flanking intron sequences of the panel of PFIC (NextGeneDx®) were done (Instituto de Medicina Genómica S.L., Valencia, Spain). PCR products were prepared with the kit Nextera XT (Illumina) and submitted to the DNA sequence with the MiSeq sequencer (Illumina). The resulting data were analyzed bioinformatically and checked by Sanger sequentiation.

Results

A total of 7 patients (5 males) had a heterozygous mutation of ATP8B1, ABCB4 or ABCB11 (Table 2). The sequence analysis revealed two missense mutations in heterozygosis in ATP8B1 (FIC1) in patient 1: c.1367C > T (p.Thr456Met) previously described in relation with PFIC1 in homozygosis, and c.3087C > A (p.Asn1029Lys) which affects a very conserved locus and probably implies a pathogenic significance. In two patients (patient 2 and 3), defects in ABCB11 (BSEP) were found: both had the defect c.1907A > T (p.Glu636Val); although the same mutation in homozygosis with another amino acid had been described to cause PFIC2, no reports in the literature of this defect exists. Patient 2 showed another pathogenic mutation c.1308 + 1G > T in ABCB11 and patient 3 showed a mutation in ABCB4, c.3715C > T (p.Arg1239Cys), described with a very low frequency so of uncertain significance. Five patients (patients 3-7, patient 3 already referred) had mutations in ABCB4 (MDR3): patient 4 showed 2 missense mutations not previously described c.1A > T (p.Met1Ile) affecting an initial reading codon and c.784dupG (p.Ala262Glyfs*31) involving an early stop codon. This patient also had an uncertain missense mutation in ABCB11 c.1331T > C (p.Val444Ala), but he behaved as an MDR3 defect, presenting with gallstones previous to diagnosis. The other three patients showed one ABCB4 mutation in heterozygosis, with no other mutations found: patient 5 showed an unknown missense mutation c.3508-3C >G that affects the mRNA correct processing; patient 6 had a c.1769G > A (p.aRG590Gin) mutation, of uncertain meaning but probably related to LPAC syndrome; patient 7 showed a mutation c.3371_3372insT in heterozygosis described in homozygosis in PFIC3.

Referring to family history, two patients (3 and 4) had a background of gallbladder stones, both showing one mutation in gene ABCB4. The mother of patient 3 had suffered intrahepatic cholestasis of pregnancy (ICP) with gestational cholestasis and mild hepatitis, also described in ABCB4 defects. Two patients (2 and 3, cousins) shared the mutation that affects BSEP, so this may be the cause that one of them does not share the family history of gallbladder stones. Moreover, these two related patients were both diagnosed with Celiac Disease.

The median age of onset was 5.5 years (1 month–15 years) with a median time of follow-up of 6 years (1 year–11 years). Patients with disease-causing variants in ABCB11 had shown an earlier onset (1 and 4 months old). The initial presentation was pruritus and/or irritability in four patients; one of them associated with hipocolia and coluria. Two patients presented with laboratory alterations: hypertransaminemia after a cholecystectomy secondary to gallstones and persistent cholestasis after a chickenpox infection. In our series, the symptomatology of the patients with a BSEP defect started earlier (1 and 4 months).

Median levels of total bilirubin at the initial study were 2.72 mg/dl (0.7–11.6) with conjugated bilirubin of 1.5 mg/dl (0.2–6.5). Median levels of gamma-glutamyl transpeptidase (GGT) were 280 UI/L (11–1256), being abnormal only in 3 patients with an MDR3 defect. All cases showed increased serum aspartate aminotransaminase (AST) 156 UI/L (48–469) and alanine aminotransaminase (ALT) 272 UI/L (49–923). Bile acid serum concentration was determined in 6 patients, being high with a median of 91 μmol/L (24–306). None of the patients associated liver synthesis dysfunction.

Abdominal ultrasound was performed in all cases, evidencing asymptomatic biliary lithiasis in one (ABCB11 mutation) and mild hepatomegaly in the patient that

Table 2 Patient's genetic results.

	Gen	Mutations	Protein	Signification
1	ATP8B1	c.1367C > T	p.Thr456Met	Pathogenic
		c.3087C > A	p.Asn1029Lys	Probably pathogenic
2	ABCB11	c.1308 + 1G > T	p.-	Pathogenic
		c.1907 A > T	p.Glu636Val	Probably pathogenic
3	ABCB11	c.1907 A > T	p.Glu636Val	Probably pathogenic
	ABCB4	c.3715C > T	p.Arg1239Cys	Uncertain
4	ABCB11	c.1331T > C	p.Val444Ala	Uncertain
	ABCB4	c.1 A > T	p.Met1Ile	Probably pathogenic
		c.784dupG	p.Ala262Glyfs*31	Probably pathogenic
5	ABCB4	c.3508-3C > G	p.-	Uncertain
6	ABCB4	c.1769G > A	p.Arg590Gln	Uncertain
7	ABCB4	c.3371_3372insT	p.-	Pathogenic

debuted after chickenpox (ATP8B1 defect), being both resolved in a later ultrasound. In 3 patients magnetic resonance cholangiopancreatography was done, showing distended gallbladder in 2 of them (ABCB4 defect in both) without any disturbance in the biliary tree.

Histology revealed mild interface hepatitis and portal inflammation in the 6 patients that had the biopsy done: 4 of them also showed mild fibrosis with portal expansion. One of them had shown moderate fibrosis in a previous biopsy performed six years before in the diagnosis approach during the first cholestatic episode, showing histological improvement in the follow-up. None showed alterations in the biliary ducts or hepatocellular cholestasis. All the biopsies samples had normal immunohistochemistry for BSEP and MDR3 activity.

In terms of treatment, ursodeoxycholic acid (UDCA) was initiated in all with good response. In 2 children, cholestyramine was added to control pruritus, improving laboratory parameters and resolving the pruritus. In the evolution of the disease, 2 patients needed supplements with fat-soluble vitamins because of vitamin E deficiency. Three cases cursed with limited relapses in the context of viral infections, anesthesia or vaccines, showing irritability, pruritus or jaundice as well as elevated bilirubin and transaminases in the laboratory tests. Relapses were treated with the addition of cholestyramine and hydroxyzine for the pruritus showing a complete clinical and biochemical normalization after the episode. None of them developed growth delay, persistent pruritus or hepatic damage progression during the follow-up (Table 3).

Discussion

Genetic variants in the hepatobiliary transport proteins (FIC1, BSEP and MDR3) comprise a broad spectrum of cholestatic liver diseases from PFIC to milder forms with limited episodes of cholestasis. While mutations that cause PFIC are often placed in conserved regions of the genes that encode conserved functional domains of the corresponding transport proteins, mutations in milder forms only partially impact on the protein expression and function.[2,6] Moreover, PFIC are recessive diseases and must be inherited in homozygosis, however, heterozygous mutations in genes encoding transport proteins are known to cause these milder forms.[6] In our series, 7 patients with an unknown origin of cholestasis showed at least one potential pathogenic variant in ATP8B1, ABCB11 and ABCB4 that may cause a partial impaired function of the transport protein. Most of the defects found were missenses, which have been associated more frequently with BRIC and milder forms, while nonsense or large deletions cause a higher failure of protein production or function with severe PFIC cases.[3,9] Apart from that, the genotypic-phenotypic correlations are still unknown with high variability in the presentation.[4,10] The same mutation in homozygous can have an incomplete penetrance while in compound heterozygous could cause PFIC.[9] Dröge et al. performed DNA sequencing in 427 patients with cholestasis phenotype identifying 25 cases with just one heterozygous disease-causing variant postulating the contribution of other mutations or epigenetic factors.[6] In our series, 3 symptomatic patients showed a unique mutation in ABCB4, this might be due to other mutations outside the sequenced areas (regulatory sequences, genes involved in transcription or control of protein trafficking), mutations in other genes or epigenetic factors that may contribute to the development and severity of the disease.[1,6,11] One of our patients showed the mutation p.Val444Ala (ABCB11) which has been described in DIC and more severe ICP[3,6]; this patient also has a compound mutation in heterozygosis in ABCB4 and behaves as an impairment of MDR3 function and fulfills the L-PAC criteria (younger than 40 years old with gallbladder stones that recurrence after the cholecystectomy surgery).[12]

In pediatric patients, the onset of milder forms, although very variable, usually occurs later than in PFIC, presenting some of them in adulthood, probably related to the less severe evolution of the disease.[13] Nevertheless, presentation is generally earlier in FIC1 and BSEP defects compared to MDR3 mutations,[6,14] with cases starting in the neonatal period with prolonged jaundice and cholestasis.[15] Patients with BRIC2 (defect in ABCB11) have been reported to have their first episode before 6 months of life.[16,17] Patients with ABCB4 (MDR3) defects are often diagnosed in adolescence or early adulthood, with cholesterol gallbladder gallstones or secondary to the increase of female sex hormones due to pregnancy or contraceptive drugs in women (ICP).[8,18] However, in our series, the onset in the MDR3 defects was

Table 3 Patient's characteristics and laboratory results in the onset of the disease.

	Gen	Family history	Age of onset	Clinic presentation	Biliary lithiasis	Histology	Treatment	Follow up (years)	Relapses
Patient's characteristics									
1	ATP8B1		5 years	Cholestasis after chickenpox		–	UDCA	1	
2	ABCB11		4 months	Pruritus Irritability	Yes	Inflammation Fibrosis (F1)	UDCA Cholesty ramine	10	
3	ABCB11 ABCB4	Gallstones ICP	1 month	Pruritus Irritability		Inflammation Fibrosis (F1)	UDCA Cholestyramine Fat soluble vitamins	6	Anesthesia
4	ABCB11 ABCB4	Gallstones	15 years	High transaminases	Yes	Inflammation Fibrosis (F1)	UDCA	4	
5	ABCB4		8 years	High transaminases		Inflammation	UDCA	7	
6	ABCB4		12 months	Pruritus		Inflammation	UDCA	3	Vaccines Viral infection
7	ABCB4		6 months	Pruritus		Inflammation Fibrosis (F1)	UDCA Fat soluble vitamins	11	Viral infection

Patient	Gen	Bilirrubin (mg/dl)	Conjugated bilirubin (mg/dl)	ALT (UI/L)	AST (UI/L)	GGT (UI/L)	Bile salts (mg/L)
Laboratory results in the onset of the disease							
1	ATP8B1	11.6	6.5	101	71	20	–
2	ABCB11	1.57	0.41	49,2	48	13.2	200
3	ABCB11/ABCB4	1.83	1	56	85	11	306
4	ABCB11/ABCB4	0.7	0.4	449	212	1246	48.5
5	ABCB4	0.3	0.2	94	69	410	24
6	ABCB4	2.4	1.6	234	143	14	58
7	ABCB4	0.7	0.7	923	469	250	100

very variable, including a wide range of ages, probably determined by the mutation location and its protein expression.

As in PFIC, milder forms are hereditary and recessive but we found only two patients with family history, all related to ABCB4 defects as gallbladder stones, already described in the literature,[18,24] and ICP.[1,5] This finding supports the theory of the great phenotypic variability and the poor genotype correlation of this entity.

Clinical presentation is also very variable.[19] As we report, the disease usually presents as intermittent episodes of pruritus and jaundice that can associate hepatomegaly and resolve spontaneously.[20–23] Only one patient, a 15-year-old boy with a defect in MDR3, associated a major complication: gallbladder stones. A possible explanation of this difference may be the age, which was much lower in the rest while gallbladder cholesterol stones in LPAC syndrome are reported in mild-age.[8,18]

In terms of diagnosis, a high index of suspicion is needed: a story of repeated episodes of pruritus with high transaminases that normalize after trigger cessation in absence of other possible explanations, may increase the suspicion of this entity. In our experience, complementary studies were performed because of irritability or pruritus with abnormal liver parameters or after the accidental finding of hypertransaminemia, but the high suspicion after ruling out other causes led us to ask for a genetic test.

As is well known, and it is presented in our patients, in the PFIC3, MDR3 is a phospholipid translocator responsible for the secretion of phospholipids and neutralization of the detergent effect of the hydrophobic bile salts. MDR3 dysfunction results in toxicity on bile ducts that leads to cholestasis with high serum levels of GGT.[1,6] This increase in the GGT was also seen during the episodes of cholestasis in our patients with heterozygous mutations in ABCB4, but it normalizes between relapses. Conversely, in PFIC1 and 2, the FIC1 and BSEP defects lead to reduce bile salt levels without toxicity on the biliary epithelium, thus these patients show normal GGT levels.[1,6] One of our patients (number 3) had a mutation in both ABCB11 and ABCB4 genes, behaving as a defect in BSEP with normal GGT. Other alterations that are found during flares are high transaminases, bilirubin and bile acids in serum.

Liver biopsies performed during flares have shown hepatocanalicular cholestasis without fibrosis, while histology in asymptomatic periods is usually normal.[2] The immunochemistry shows normal expression for BSEP and MDR3, which supports the theory of a functional protein defect.[1,24] A therapeutic approach should be established based on the severity and residual activity of the transporter. Although UDCA has shown to be more effective in ABCB4 deficiency, even resolving advanced fibrosis,[25–27] in the low GGT defects, can also improve pruritus and liver enzymes.[26] Our patients were treated with UDCA obtaining good results. Rifampicin acts as an enzyme inducer, reducing itching with greater efficacy than UDCA.[26] Recently, the histone deacetylase inhibitor 4-phenyl butyric acid (4-PBA) has shown its efficacy as a PFIC treatment.[15,21] Cholestyramine is the first approach for alleviating cholestatic pruritus, but has no direct effect on cholestasis.[10] In severe progressive cases or patients with frequent or debilitating flares, partial biliary diversion with interruption of bile salts enterohepatic circulation can be considered.[10,28,29] In progressive severe cases, a liver transplant may be the final treatment.[26]

Our patients showed limited new episodes without liver disease progression, despite the diagnosis age. Nevertheless, there are cases reports published with evolution to PFIC by the time despite an initial history of auto limited episodes of cholestasis, which supports the understanding of this pathology as a continuum spectrum from milder forms to permanent cholestatic and severe diseases as PFIC.[7,8,14,16,17,22,30]

Conclusions

Heterozygous mutations in genes involved in hepatocellular transport may lead to milder forms of intrahepatic cholestasis with great phenotypic variability and poor genotypic correlation. It is an infrequent entity, probably secondary to its poor knowledge and low index of suspicion leading to a delay in the diagnosis in adult age. Patients with repeated episodes of high transaminases or pruritus after a trigger may lead us to suspect the diagnosis, confirmed by the genetic test. In our series, choleretic drugs as UDCA have shown to be effective. We should be aware of the risk of progression of the disease even if the mutation and the phenotype correspond to a milder form.

Ethics approval

Until September 2020 in our Hospital the institution ethics approval was not obligatory for observational retrospective studies as long as informed consent was obtained from the guardians. This original work was done before this statement was approved.

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Authos' contribution

Beatriz Mínguez and Cristina Molera have participated in the conception, design of the work and literature research. Beatriz Mínguez, Cristina Molera, Ruth García Romero, Gemma Colomé and Loreto Martorell have collected data and analyzed it. All the authors mentioned and Javier Martín de Carpi has participated in the interpretation of the results, writing the draft of the manuscript and its corrections. All the authors agreed with the content and gave consent to publish the work.

Consent to participate

Informed consent was obtained from legal guardians.

Availability of data and material

The data and material have been retrospective obtained from the clinical records after the patients diagnose and informed consent. This study was presented as an oral communication in the XXVI National Congress of the Spanish Society of Pediatric Gastroenterology, Hepatology and Nutrition (SEGHNP) in Santander, Spain and it was accepted as a poster in the 6th World Congress of ESPGHAN 2020 in Copenhagen, Denmark.

Code availability

We did not use any software.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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