Description of two new \( \text{ABCB11} \) mutations responsible for type 2 benign recurrent intrahepatic cholestasis in a French-Canadian family

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Benign recurrent intrahepatic cholestasis (BRIC) is a rare cause of hereditary cholestatic liver disorders, and is sometimes difficult to differentiate from progressive familial intrahepatic cholestasis (PFIC). The main difference between BRIC and PFIC is their clinical and biochemical evolution. BRIC is characterized by a benign course, with recurrent episodes of cholestasis and complete normalization of liver biochemistry between each episode (1). PFIC is characterized by the progressive appearance of pruritus, growth failure, coagulopathy and, eventually, liver failure at a relatively young age (2-4). However, the boundary between these two entities can sometimes overlap, which makes precise, early diagnosis difficult (5). Therefore, they are often considered as a phenotypic continuum with variable course. In recent years, several proteins involved in biliary physiology, such as canalicular transporters, have been found to harbour mutations that were later associated with BRIC and PFIC syndromes. Therefore, based on these findings, redefinition of these disorders is currently in progress (6,7). Therefore, it is not surprising that mutations in the \( \text{ABCB11} \) gene revealed two previously unreported mutations that predict the absence of expression of the protein. The clinical presentation of the current cases are discussed, as are the differential diagnosis and genetic characteristics of the hereditary cholestatic disorders, overemphasizing the possibility of making a definite genetic diagnosis.

**Key Words:** \( \text{ABCB11} \); Benign recurrent cholestasis

Benign recurrent intrahepatic cholestasis is a rare clinical entity that is caused by mutations in the canalicular transport genes. The present report describes two individuals from the same family whose symptoms were typical of the clinical characteristics of type 2 benign recurrent intrahepatic cholestasis. Sequencing of the \( \text{ABCB11} \) gene revealed two previously unreported mutations that predict the absence of expression of the protein. The clinical presentation of the current cases are discussed, as are the differential diagnosis and genetic characteristics of the hereditary cholestatic disorders, overemphasizing the possibility of making a definite genetic diagnosis.

**CASE PRESENTATION**

The present report describes the discovery of two previously unreported mutations in the \( \text{ABCB11} \) gene identified in two Caucasian sisters previously diagnosed with BRIC. The history of the clinical development of their syndromes is described.

Both sisters were diagnosed in early childhood – one in the neonatal period and the second at nine months of age. Intractable intermitten pruritus and jaundice were the dominant clinical features. Ursodeoxycholic acid was prescribed soon after diagnosis. Pruritus was controlled with rifampicin and/or cholestyramine. During the 20 to 25 years of follow-up, neither patient exhibited signs or complications of chronic liver failure; in fact, the liver always appeared normal when examined by ultrasound. Therefore, liver enlargement and splenomegaly were never detected. Of note, one of the two sisters developed acute pancreatitis at a young age, which was judged to be secondary to biliary sludge without, however, any evidence of cholestasis. This same patient was also diagnosed with unilateral deafness of unexplained etiology at a very young age.

In the course of the evolution of their disease, these patients developed two to three episodes of clinically significant jaundice of approximately six months to 1.5 years duration each. Interestingly, the duration and severity of each occurrence appeared to be longer and more severe. Moderate hepatocyte injury was observed each time, and was characterized by a two- to eightfold increase in the upper limit of normal (ULN) levels of serum aspartate aminotransferase and alanine aminotransferase, three times the ULN for alkaline phosphatase levels, and bilirubin levels reaching more than 200 µmol/L. However, gammaglutamyl transpeptidase activity always remained normal. Both patients experienced high blood levels of low-density lipoprotein and low levels...
of high-density lipoprotein cholesterol in the blood during the episodes as well (total cholesterol 7.68 mmol/L and 5.68 mmol/L; low-density lipoprotein cholesterol 6.3 mmol/L and 4.0 mmol/L; and high-density lipoprotein cholesterol 0.34 mmol/L and 0.66 mmol/L). These episodes were most often triggered by mild upper respiratory tract infections; however, some occurred without a clear triggering factor. Of note, one occasion, cholestasis appeared in the first two weeks following the introduction of minimal doses of oral contraceptives, suggesting that both events were linked. Neither of the sisters have become pregnant. Overall, these two patients were subjected to multiple liver biopsies during their childhood. Results showed evidence of canalicular cholestasis, mainly located in the centrilobular area, without any signs of biliary duct damage, proliferation or fibrosis. In between episodes, both sisters reported chronic mild pruritus.

To assess the genetic transmission of their syndrome, the phenotype of the parents of the two sisters was investigated and a family pedigree was created. Both parents were of French-Canadian descent and had been living in a small area (±100 km²) of the Lanaudière region (Quebec) for at least the past four generations, but they were not related. They had another unaffected 28-year-old female child.

The mother (55 years of age) had no history of jaundice, cholestasis complications during her pregnancies or mutations in the hemochromatosis HFE gene. Her liver biochemistry was completely normal. She was only diagnosed with osteoporosis. On the other hand, nine of her 11 brothers and sisters underwent gallbladder surgery in the past. The father (57 years of age) was diagnosed with hereditary hemochromatosis 20 years previously; four of his eight brothers and sisters were also diagnosed with the disease. The condition is currently well controlled biochemically with phlebotomy treatment. A physical examination showed mild joint deformities without darkening of the skin or liver enlargement. His liver biochemistry was normal, except for a slightly elevated total bilirubin level (less than two times ULN).

Genetic screening revealed that he was homozygous for the C282Y mutation in the hemochromatosis HFE gene. Noteworthy, the father harboured the 830 C→A (A277E) mutation. Therefore, the daughter (40 years old) was found to have the 390 G→T (G130G) mutation, while the father was found to harbour the exact two same mutations. Sequencing of polymerase chain reaction products generated from genomic DNA extracts of peripheral blood cells drawn from one of the two sisters. A splice mutation (390 G→T [G130G]) was found on one allele, while a missense sequence variant (830 C→A [A277E]) was found on the other allele. Mutation nomenclature is based on the recommendation of the American College of Medical Genetics designating the initial ATG start codon as +1 using the reference sequences NT_005403 and NM_003742. The genetic codons in which these mutations are localized are well conserved in mammals according to DNA alignment software. The splice mutation is located at a well-identified splice site that is translated in the canalicular domain of the protein (9). The other sister was found to harbour the two exact same mutations. Sequencing of ABCB4 and ATP8B1 genes was also performed for one of the two sisters, with no mutations identified. Therefore, it was decided that these cases be classified as type 2 BRIC (BRIC-II) (Table 1).

Finally, he suffers from partial hereditary deafness, which required stapes prosthetic replacement. Four of his eight brothers have the same disorder.

Mutations in the ABCB11 gene were investigated by direct sequencing of polymerase chain reaction products generated from genomic DNA extracts of peripheral blood cells drawn from one of the two sisters. A splice mutation (390 G→T [G130G]) was found on one allele, while a missense sequence variant (830 C→A [A277E]) was found on the other allele. Mutation nomenclature is based on the recommendation of the American College of Medical Genetics designating the initial ATG start codon as +1 using the reference sequences NT_005403 and NM_003742. The genetic codons in which these mutations are localized are well conserved in mammals according to DNA alignment software. The splice mutation is located at a well-identified splice site that is translated in the canalicular domain of the protein (9). The other sister was found to harbour the exact two same mutations. Sequencing of ABCB4 and ATP8B1 genes was also performed for one of the two sisters, with no mutations identified. Therefore, it was decided that these cases be classified as type 2 BRIC (BRIC-II) (Table 1).

Finally, to evaluate the genetic phase transmission of these mutations, the ABCB11 gene in both parents was sequenced. The mother was found to have the 390 G→T (G130G) mutation, while the father harboured the 830 C→A (A277E) mutation. Therefore, the daughters’ genotype was transmitted from both parents.

**DISCUSSION**

Synthesized and recycled bile acids are transported from hepatocytes across canalicular membranes against a concentration gradient driven by an ATP-dependent pump. This ATP-dependent transporter is encoded by the gene ABCB11, which is also known as the BSEP. The
BSEP is expressed primarily in the liver, but is also expressed at lower levels in other organs such as human testis (10), brain cortex (11), placenta (12), small and large intestines (13), and kidneys (14). The functional role of this protein in nonhepatic tissues is not completely clear. Autosomal recessive mutations in this gene are responsible for rare hereditary forms of cholestatic liver disorders known as BRIC-II/PFIC-II. To date, approximately 150 mutations in the ABCB11 gene have been identified (9,15-19). These mutations include insertions, deletions, splice or nonsense mutations of the gene leading to reduced expression of the protein in hepatocytes, thus reducing or completely inhibiting its activity. Among the two most common mutations in individuals of European descent are the E297G and D482G mutations, which collectively account for approximately 58% of all cases (9).

In the present report, we described two young sisters who shared two previously unreported mutations in ABCB11; however, even if these mutations are expected to inactivate the product of this gene, it would require protein expression studies to confirm it. The sisters inherited these genes from their parents in a genetic trans phase because each of the two parents is heterozygous for one of the identified mutations. As previously mentioned, BRIC is inherited in an autosomal recessive pattern, and parents of a proband are generally obligate carriers of the disease-causing mutation. Interestingly, the probability of observing, as was the case in the present study, two sisters with the same mutation in an autosomal recessive pattern is only 6%.

Clinically, both sisters presented with bouts of jaundice that lasted approximately six months to 1.5 years, and were characterized by a mixed pattern of hepatocellular injury with normal levels of gamma-glutamyl transpeptidase. As documented previously, minor viral infections have been responsible for these acute episodes. Interestingly, each new exacerbation seems to be more severe in intensity and in duration over time. However, neither of the sisters have shown evidence of liver failure to date, with serum albumin and coagulogram levels remaining close to normal. For the moment, this benign evolution seems to be consistent with the diagnosis of BRIC-II.

A strong penetrance for cholestasis seems to be present in this family, especially for the splice mutation variant G130G. Several mutations have been shown to increase the risk of cholelithiasis in cases of nonfamilial BRIC. Our observation is based only on a family review and not on genetic sequencing; therefore, the results should be interpreted with caution. Previous reports (20) have linked mutations in ABCB11 and ABCB4, along with ABCG8 of the canicular ABCG5/ABCG8 cholesterol transporter with gallstone disease. This can be easily understood considering that these transporters carry compounds that have all been implicated in the formation of gallstones. Furthermore, a coding variant in ABCG8 has been documented to be significantly associated with gallstones in large patient panels (21,22); however, no ABCB11 variants have been implicated in gallstone formation outside the context of BRIC-II (23).

Others have reported a correlation between the type of mutation and disease severity. Indeed, mutations that were predicted to affect expression or function of the gene protein to a greater extent, such as frameshift, nonsense and large deletions, were detected more frequently in patients with progressive disease (3). According to this hypothesis, the new mutations described in the present report could be sufficient to cause acquired gallstone disease in heterozygous patients, and BRIC-II in homozygotes. One intriguing finding, however, is that the two homozygote cases have not presented with gallstones to date.

It was also surprising to find evidence of probable extrahepatic manifestations of the disease. Indeed, deafness is believed to be exclusively observed in BRIC-II/PFIC-I disease (24,25). In our case study, one of the two daughters has a hearing problem of unclear origin. We do not believe that the history of stapes disorder in the father’s family was linked to the ABCB11 mutations because this has not been previously documented, and because it was not the diagnosis in the affected daughter. Finally, the history of acute nonlithiatic pancreatitis in the same case is also original because this type of extrahepatic manifestation is, again, more characteristic of BRIC-I even though it has also been rarely observed in BRIC-II (4,24).

Aside from genotyping, it is also possible to perform immunohistochemical analysis of the BSEP protein on liver biopsy specimens (9,18). This may enable a more rapid diagnosis if the technique is already mastered in the local laboratory. Despite generally good agreement between the immunohistochemical evaluation of the BSEP protein and the presence of ABCB11 mutations, discrepancies exist, particularly with certain genotypes (18). Finally, transport studies have also been performed to test the functional capacity of the mutated protein in vitro (18,26).

Clearly, more data need to be accumulated to better characterize the clinical spectrum of these rare disorders. This is especially important to be able to more effectively predict the clinical evolution of these patients. In particular, we acknowledge that hepatocellular carcinoma has been described in individuals harbouring ABCB11 mutations (9,27). Furthermore, it is possible to observe some BRIC patients who eventually evolve toward liver failure and subsequently adopt a PFIC phenotype (28). Finally, the identification of such new mutations in a French-Canadian family raises the possibility that these mutations are specific to this particular population, as has been described in other areas of the world (29). With the advent of molecular genetic testing, it is now possible to identify and categorize these individuals more clearly.

REFERENCES

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