CASE REPORT

A Novel Truncation Mutation in *ATP8B1* Gene in Progressive Familial Intrahepatic Cholestasis

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Background*:* Progressive familial intrahepatic cholestasis has been only infrequently reported from India. **Case characteristics***:* An Indian girl with progressive cholestatic liver disease beginning during infancy, normal gamma-glutamyl transpeptidase levels, parental consanguinity, positive family history and a fatal outcome. **Observation***:* A novel, homozygous mutation (c.[589_592inv;592_593insA]) in *ATP8B1* gene, with a markedly truncated protein (p.[Gly197LeufsTer10]) was found. **Message**: The novel mutation found expands the spectrum of genetic variations associated with progressive familial intrahepatic cholestasis.

Keywords: *Diagnosis, Genetic variation, Neonatal cholestasis, Protein truncation*.

Progressive familial intrahepatic cholestasis (PFIC), a genetic disorder, accounts for 10%-15% of cholestatic liver disease in children [1]. The condition is classified into three types, namely PFIC1, PFIC2 and PFIC3, related to mutations in *ATP8B1* gene, *ABCB11* gene and *ABCB4* gene, respectively [2-4]. PFIC1 and PFIC2 manifest during infancy and progress to end-stage liver disease during early childhood, whereas the onset of PFIC3 is often delayed. Despite some phenotypic differences, differentiation is based primarily on genetic findings.

Some individual cases [5,6] and small case series [7,8] of suspected PFIC have been reported from India, but there are no data on genetic variations in this disease.

CASE REPORT

A 4.5-month-old girl, born at term to second-degree consanguineous parents, presented with jaundice and pruritus for one month. She was fourth in birth order with one intrauterine death, a 8-year-old healthy sister, and one sister who had died at 5 years of age of decompensated liver disease (which began with jaundice and pruritus at 3 months of age). The mother reported intense itching during third trimester in each pregnancy.

At presentation, she weighed 5.5 kg (5^{th}-10^{th} centile), was 59 cm long (10^{th}-25^{th} centile), and had mild icterus and a soft liver palpable 3 cm below the costal margin. Conjugated hyperbilirubinemia (total serum bilirubin 7.0 mg/dL, conjugated 5.0 mg/dL), high alkaline phosphatase (630 U/L; reference range for adults 35-150) normal serum alanine and aspartate aminotransferases, and low-normal GGT activity (8 U/L) were seen. Serum albumin level (3.7 g/dL) and prothrombin time (international normalized ratio = 1.1) were normal. Facility for serum bile acid levels was not available. Ultrasonography showed normal biliary system. Percutaneous liver biopsy showed maintained lobular architecture, minimal portal inflammation and bland cholestasis; immunohistochemistry was not done. In view of cholestasis with normal GGT, family history of similar illness, history of cholestasis during pregnancy in the mother, and consanguinity, a diagnosis of PFIC was made.

Treatment with ursodeoxycholic acid, rifampicin and cholestyramine, vitamin supplementation, and diet rich in medium-chain triglycerides did not provide any symptomatic improvement. The parents declined surgical treatment. Over time, her disease worsened, ascites and coagulopathy appeared, and serum albumin levels declined. She died at home at the age of 4 years, with worsening ascites and encephalopathy.

All the coding exons of *ATP8B1*, *ABCB11* and *ABCB4* genes were amplified [2-4] and sequenced. This revealed a novel, homozygous variation in exon 7 of the *ATP8B1* gene (c.[589_592inv;592_593insA]; ***Fig.* 1*a-b***), with replacement of a 4-nucleotide sequence (GGAG) by five nucleotides CTCCA. It was predicted to lead to a truncated protein with 205 amino acid instead of a normal protein with 1251 amino acids (***Fig.* 1c**). The *ABCB11* gene also showed a non-synonymous variation in exon 13

(c.1331T>C; rs2287622), which was predicted as unlikely to adversely affect the protein function (*http://snps.biofold.org/meta-snp/index.html*).

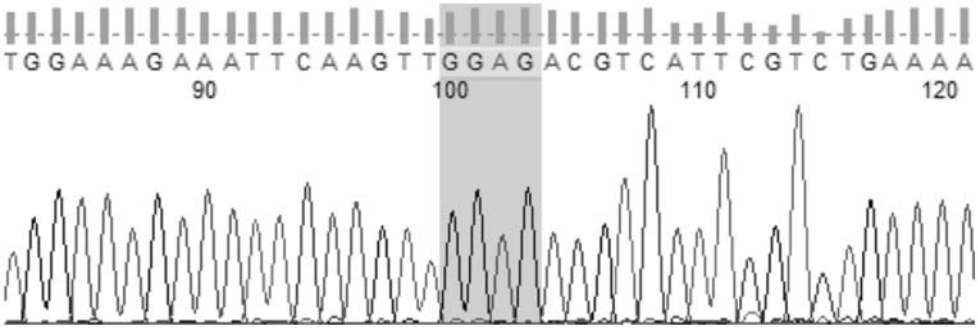
In addition, there were some previously-known synonymous variations in the *ATP8B1* (c.696T>C [rs319438] and c.811A>C [rs319443]), ABCB11 (c.3084A>G [rs497692]) and *ABCB4* (c.711A>T [rs2109505]) genes. Both the parents had the c.[589_592inv;592_593insA] frame-shift variation in heterozygous state.

DISCUSSION

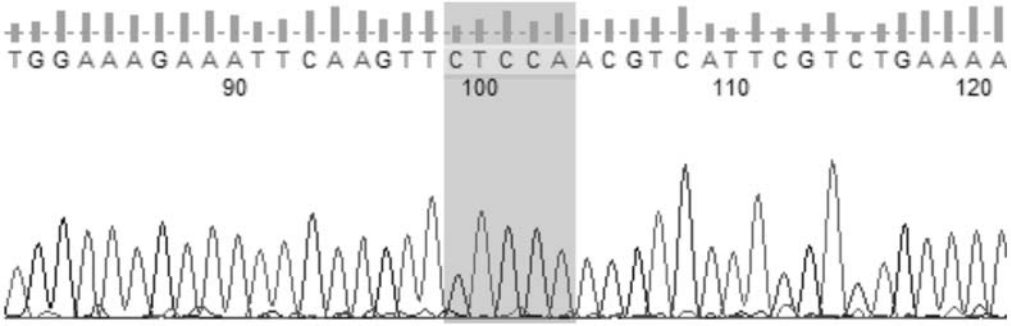
This report describes an Indian child who had liver disease starting in infancy, with clinical features typical of PFIC, and a strong family history of liver disease. Normal GGT levels indicated that PFIC3 was unlikely. However, in the absence of extrahepatic features, it was not possible to distinguish whether she had PFIC1 or PFIC2. The detection of a major mutation in exon 7 of the *ATP8B1* gene, which was expected to severely disrupt the structure of this gene's product confirmed that this patient had PFIC1.

Data on PFIC from India are limited [5-8]; there are no published data from other South Asian countries. Genetic abnormalities underlying this disease have not been studied. In our patient, we did consider the

(*a*)



(*b*)



(*c*)

Exon 7 of PFIC1 (Reference)

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191                     197 198                             206
TGG AAA GAA ATT CAA GTT GGA GAC GTC ATT CGT CTG AAA AAA AAT GAT
Trp Lys Glu Ile Gln Val Gly Asp Val Ile Arg Leu Lys Lys Asn Asp
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Exon 7 of PFIC1 (Patient)

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191                     197 198                             206
TGG AAA GAA ATT CAA GTT CTC CAA CGT CAT TCG TCT GAA AAA AAA TGA
Trp Lys Glu Ile Gln Val Leu Gln Arg His Ser Ser Glu Lys Lys End
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FIG. 1 *Sequencing chromatograms from a part of exon 7 of ATP8B1 gene from a healthy person showing the reference sequence (a) identical to GenBank accession number NM_005603.4): and our patient (b) show a c.589_592inv; 592_593insA change. This genomic change leads to a frame shift with a change in the amino acid sequence and premature truncation of the protein after amino acid location 205). Underlining indicates the nucleotides and amino acids that have changed (c).*

possibility of biliary diversion and liver transplantation. In the literature, symptomatic relief after biliary diversion has been observed in only a subset of patients, and its occurrence cannot be predicted in advance [1]. In PFIC1, though liver transplantation reliably reverses cholestasis, other extrahepatic manifestations, such as diarrhea and hepatic steatosis may worsen or appear when not already present, by permitting a larger amount of bile salts to reach the gut [9]; short stature also does not respond. In PFIC2 patients with severe disease who need transplantation, the procedure is often complicated by disease recurrence; this is believed to be related to development of antibodies to the PFIC2 protein present in the transplanted organ [10].

In our patient, we were able to show the presence of a novel mutation in the *ATP8B1* gene. This gene encodes a 1251-amino acid long protein which is involved in the transport of aminophospholipids across cellular membranes. Several mutations in this gene have been described, including non-sense mutations that alter the amino acid sequence of the protein, small insertion or deletion mutations that induce frameshifts, in-frame deletions of variable size, and mutations that may disrupt splicing [2]. Genotype-phenotype correlation shows that missense mutations are more common in a condition known as benign recurrent intrahepatic cholestasis, a mild disease, whereas nonsense, frame-shift, and large deletion mutations are more common in patients with PFIC [2]. The novel mutation found in our patient led to a markedly truncated protein which would lack the active domain and hence be non-functional; this may explain the severe disease in this kindred.

Genetic analysis in PFIC is important for accurate diagnosis and possibility of prenatal diagnosis during subsequent pregnancies in the family. Such analyses, by providing information on mutations prevalent in Indian patients with PFIC, may permit a directed testing for the common mutations rather than using sequencing for several exons.

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Contributors: AS: did the laboratory work and data analysis; SA: did the bioinformatic analysis; UP: was involved in clinical work up of the patient; RA: conceived the work, supervised laboratory work and data analysis, and will be the guarantor. All the authors were involved in planning of this work, writing of the report, and approval of the final version of the manuscript.
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