A novel ABCB11 mutation in an Iranian girl with progressive familial intrahepatic cholestasis

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Introduction

Progressive familial intrahepatic cholestasis (PFIC), known as a group of heterogeneous autosomal recessive disorders, hit liver hepatocellular bile salt secretion as a cholestasis of hepatocellular origin appearing in infants and leading to death from liver failure. Three types of PFIC have been defined from the hepatocellular transport system defects and related genes, which involved in bile formation.[1-3] Main clinical manifestations include cholestasis, pruritus and jaundice. These patients usually develop fibrosis and end-stage liver disease before adulthood.[4] PFIC type 1 is caused by mutations in ATP8B1 (chromosome 18q21), which encodes FIC1 protein. PFIC type 2 is identified by mutations in ABCB11 (chromosome 2q24), which encodes bile salt export pump (BSEP) and defects in ABCB4-encoding the multi-drug resistant 3 (MDR3) protein – impair biliary phospholipid secretion cause PFIC3. Both PFIC1 and PFIC2 are caused by impaired bile salt secretion appearing in first months of life but PFIC3 is caused by impaired biliary phospholipid secretion and may manifest later.[1,2]

Case Report

A nine-year-old girl with cirrhosis was referred for genetic counseling. The patient was affected by icterus and pruritus from newborn period. Her brother and sister displayed the same symptoms; they died at the age of 1.5 and 7 years, respectively. Her parents had a non-consanguinity marriage. The patient’s serum biochemical analysis indicated high in total and direct bilirubin and S.G.P.T (ALT). The copper level was raised in serum biochemical result. In CT scan examination, liver and gallbladder had normal sizes but spleen was significantly enlarged. Microscopic examination from a liver biopsy specimen showed significant pathological characterizations of cholestasis. Hepatocytes indicated extensive pseudo acinar transformation; ballooning degeneration, cholestasis and bile plague. Portal tracts contain moderate lymphocytic infiltration and also

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necrosis in focal confluent. Fibrosis expansion of portal tracts with marked bridging and focal bile duct damage were observed. From the pathological examination of the hepatocytes and clinical demonstrations, the changes were compatible with progressive familial intra-hepatic cholestasis type 1 or 2. The liver biopsy result from the expired sister had the same pathological characteristics of the proband and ended with cirrhosis.

Pathological study

The diagnosis of the kind of PFIC can be difficult because PFIC type 1 has initial clinical and laboratory findings similar to those of PFIC type 2.\(^1,2\) Pathological features may differ. In PFIC type 1 bland canalicular cholestasis with variable fibrosis is found. In PFIC type 2, variable features include canalicular cholestasis and a neonatal hepatitis pattern, with hepatocellular swelling and giant cell transformation.\(^1,2\) Immune staining is a useful diagnostic tool for PFIC type 2 since most patients with ABCB11 mutations and hepatobiliary disease of onset in infancy have no canalicular BSEP expression.\(^5\) So pathological study was performed on proband. Hepatocellular swelling with multinucleated canalicular and hepatocellular cholestasis, portal lymphocytic infiltration and fibrosis, newly formed liver nodules, bridging necrosis, bile duct proliferation, giant cells and micro vesicular fat droplet can be seen in proband liver biopsy [Figure 1].

Mutation Analysis

Mutation analysis was performed on DNA of the patient and both parents. The genomic DNA was extracted from peripheral blood leukocytes and ABCB11 of all 27 coding exons and intron/exon borders were screened by intronic oligonucleotide primers as reported previously\(^6\) and direct sequencing of PCR products. A novel homozygous mutation 3593-A > G was detected in exon 25 [Figure 2]. This mutation leads to the missense mutation (His1198Arg). There is a small physicochemical difference between His and Arg, but the amino acid histidine at codon 1198 is highly conserved between species (up till C. elegans) and isoforms (ABCB 1, 4, 5, 6, 7, 8, 9, 11). PolyPhen predicts a probably damaging


effect (PSIC score difference: 3.349) and SIFT also predicts that this substitution could affect protein function. Parents were heterozygote for this mutation.

**Discussion**

To the best of our knowledge, this is the first case report of a patient with typical clinical characterizations of pathological PFIC type 2 in Iran. The patient had the clinical presentation of progressive cholestasis toward the cirrhosis like her two previous siblings expired at the age of 1.5 and 7 years old had affected to the PFIC type 2. The primary diagnosis of the disease was between PFIC type 1 or 2. The most clinical features of the patient and her previous sister are in common. The first clinical characterizations of disease appeared at the early stage of infancy with jaundice incidence. Alanine transferase (ALT) and total and direct bilirubin and serum copper were elevated in serum biochemical result. Liver and gallbladder had normal sizes but spleen size was significantly enlarged. Bile duct proliferation, inflammation, fibrosis or cirrhosis is common features in liver histology of patients with PFIC type 1 and 2. Pathological examination indicated pseudo acinar transformation, ballooning degeneration, cholestasis and bile plague. Portal tracts contain moderate lymphocytic infiltration and also necrosis in focal confluent. Fibrosis expansion of portal tracts with marked bridging and focal bile duct damage were observed. In PFIC type 2, the liver histology shows more inflammatory activity than in PFIC type 1 with giant cell transformation and lobular portal fibrosis. To date, more than 100 mutation in \( ABCB11 \) gene have been identified.\[^5,7,8\] Severe phenotypes are often associated with mutations that lead to premature protein truncation or failure of protein production. Insertion, deletion, nonsense, and splicing mutations result in damaging effects, and patients who have clinical PFIC associated with such mutations exhibit little or no BSEP expression in hepatocyte canalicular.\[^2,5\]

In Asian patients, few reports of mutations in PFIC type 2 exist\[^9,10\] Goto et al.\[^8\] have reported four mutations in \( ABCB11 \) gene, predicated to yield V330X, R487H, R575X and E636G in two Japanese PFIC patients. Chen et al.\[^10\] have reported seven BSEP mutations (M183V, V284L, R303K, R487H, W493X, G1004D and 1145delC) in four PFIC patients of Chinese descent; none of these mutations has been described in Caucasian patients. But here we found a new mutation in \( ABCB11 \) gene. This mutation is a novel homozygous mutation that leads to the missense mutation (His1198Arg).

**References**