CASE REPORT

Progressive Familial Intrahepatic Cholestasis Type 3: A Novel Mutation in a Saudi Child

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ABSTRACT

Progressive familial intrahepatic cholestasis type 3 (PFIC3) is caused by defects in ABCB4 gene. Liver histology although important, but is nonspecific, and molecular genetic testing is essential for diagnosis. To report PFIC3 in a Saudi male child and determine the pathogenetic role of a novel of ABCB4 in one of them. Liver biopsy, immunohistochemical analysis for MDR3 protein expression and molecular genetic analysis were done for the patient. Liver biopsy showed extensive ductular reaction with portal and peripoortal fibrosis. Immunohistochemical analysis revealed absence of MDR3 protein expression at the canalicular pole. Molecular genetic analysis revealed a novel mutation in ABCB4: the c.1783 C > T (p.Arg595X) mutation in exon 15 in homozygous state. A novel loss-of-function mutation has been identified. Molecular genetic testing is essential and conclusive for diagnosis.

Key words: Progressive familial intrahepatic cholestasis type 3; ABCB4 gene mutations; Children; Saudi Arabia


INTRODUCTION

PFIC refers to heterogeneous group of autosomal recessive disorders of childhood that disrupt bile formation and present with pruritus and cholestasis of hepatocellular origin[1,2]. Three distinct forms are described: PFIC1 or Byler disease[3] [OMIM211600] and PFIC2 or bile salt export pump (BSEP) disease[4] [OMIM 601847], that are caused by impaired bile salt secretion and are associated with a low or normal serum gamma glutamyl transpeptidase (GGT) activity, and PFIC3 or multidrug resistance protein 3 (MDR3) disease[5] [OMIM 171060], impair biliary phospholipid (PL) secretion and is associated with a high serum GGT activity.

PFIC1 and PFIC2 usually appear in the first months of life, whereas PFIC3 occurs later in infancy, in childhood or even during young adulthood. PFIC patients usually develop fibrosis and end-stage liver disease before adulthood[1]. Death from liver failure usually occurs if LTx is not performed[6].

Liver histology in PFIC1, is characterized by canalicular cholestasis and the absence of true ductular proliferation with only peripoortal biliary metaplasia of hepatocytes. Same findings are seen in PFIC2 but the liver architecture is more perturbed, with more pronounced hepatocellular necrosis, giant cell transformation, lobular and portal fibrosis and inflammation[7-9]. In PFIC3, portal fibrosis and true ductular proliferation with mixed inflammatory infiltrate are the main characteristics[10]. Cytokeratin immunostaining confirms the strong ductular proliferation within the portal tract. At a later stage, there is extensive portal fibrosis and a typical picture of biliary cirrhosis[10].

MDR3 is expressed, almost exclusively, at the canalicular membrane of the hepatocytes. Its main role is the excretion of biliary PLs. In PFIC 3, there is malfunction of MDR3 resulting in...
cholestasis which resulted from the toxicity of bile in which detergent bile salts are not neutralized by PLs, leading to bile canalicular and biliary epithelium injuries. The absence of PLs in bile destabilize micelles and promote lithogenicity of bile with crystallization of cholesterol, leading to obstruction of small bile ducts and ductular reaction[14].

In the present study we report a Saudi child with PFIC3 in whom liver histology showed extensive ductular reaction with portal and perportal fibrosis. Genotyping revealed a novel mutations in the PFIC3 gene, ABCB4 which is considered the 1st case with this mutation in KSA and the 2nd reported in literature after that reported by Giovannoni et al[1].

CASE REPORT

SA is a 2-year-and 8-month old Saudi male child who was born to healthy first-degree Saudi cousins, at term with uneventful perinatal history and normal developmental history with negative family history of liver disease. His parents noticed generalized abdominal distension at the age of 1 year for which they sought medical advice but unfortunately was not investigated until he presented to us 4 months ago with mild jaundice and itching all over the body added to the existing chronic abdominal distension. On examination, there was tinge jaundice and hepatomegaly. Liver felt 4 cm in Rt MCL, 3 cm in midline with hepatic span 10.5 cm in the Rt MCL. It was firm in consistency with smooth surface. No splenic enlargement with unremarkable other body system review. Laboratory investigations revealed; mild cholestatic jaundice with total bilirubin 45 umol/L and direct bilirubin 35 umol/L, high transaminases with ALT: 569 IU/L (0-55) and AST: 526 IU/L (5-34), high GGT 333 IU/L (<55), and high alkaline phosphatase 1106 IU/L with normal synthetic functions. Serum total bile acids were high 185.6 umol/L (0-8) with high serum primary bile acids levels [Cholic Acid: 70.2 umol/L (10.0-24.0), Chenodesoxycholic Acid: 29.3 umol/L (29.0-46.0)], and low secondary serum bile acids levels [Deoxycholic Acid: 0.4 umol/L (2.5-15.0), and Lithocholic Acid: 0.0 umol/L (0.0-2.0)]. Phospholipid concentration was not available.

Liver biopsy was performed and was suggestive of advanced fibrosis (stage 3) and early cirrhotic changes. There was slightly altered hepatic architecture by the presence of focal bridging fibrosis, mild centrlobular fibrosis and micronodules with evidence of cholestatic hepatitis and giant cell transformation. Portal tracts showed inflammatory infiltrates with lymphocytes, polymorph nuclear leucocytes & eosinophils with bile ductular proliferation with normal small and large bile ducts with no evidence of bile ducts damage (Figure 1 A, B, C, D). In addition to histological processing, immunohistochemistry was performed for cytokekin 7 & 19 and confirmed marked bile ductular proliferation (Figure 1 D, E).

Immunohistochemistry for MDR3 protein showed complete absence of canaliculainmunostaining for MDR3 protein.

Genomic DNA was purified from peripheral blood and the coding exons 1-28 and the exon-intron boundaries of the ABCB4 gene (OMIM 171060) were amplified by polymerase chain reaction[10], and sequenced directly. The resulting sequence data were compared with the reference sequence NM_000443.3. Sequencing analysis revealed at position c.1783 in exon 15 of the ABCB4 gene, the nucleotide exchange C to T homozygous state (c.1783 C>T). This nonsense substitution truncates the protein at codon 595 (p.Arg595X), thus generating a presumably non-functional protein.

Thus our patient carries the mutation c.1783 C>T (p.Arg595X) in the ABCB4 gene in the homo-/hemizygous state.

DISCUSSION

Both the clinical and pathological findings of PFIC 3 are not confirmatory alone. MDR3 immunostaining in the liver and ABCB4 testing are essential to confirm diagnosis. PFIC3 is caused by mutations of the ATP-binding cassette subfamily B (MDR/ TAP) member 4 (ABCB4) gene, also known as MDR3. ABCB4 belongs to the superfamliy of membrane transporters that contain an ATP-binding cassette (ABC-transporters)[11] and hydrolyze ATP during translocation of a wide variety of molecules across membranes, a function that requires the concerted action of two ABC transmembrane domains and two ABC transporter domains[12]. Specifically, the domains essential for organic molecule efflux are arranged along the ABCB4 protein sequence in the amino acid ranges 57-359 (1st ABC transmembrane transporter), 394-630 (1st ABC transporter), 711-999 (2nd ABC transmembrane transporter) and 1034-1279 (2nd ABC transporter) according to Prosite database[13].

Most of the mutations identified in ABCB4 gene were described by Degiorgio and his colleagues 2007, table 1[10]. The mutation identified in this study is the 1st novel mutation reported from KSA and the 2nd in literature after the report of Giovannoni et al[1].

This mutation is predicted to generate proteins that are unable to carry out their transporter function, that is why liver immunohistochemistry did not show any canaliculainmunostaining for MDR3, suggesting that the mutant protein cannot reach the canaliculain membrane.

On the other hand, normal or slightly reduced canaliculainmunostaining does not exclude the presence of MDR3 dysfunction[14]. That is the reason why direct gene testing is mandatory[14].

The current report presented a loss-of-function mutation in a Saudi child. Although occasionally described in Saudi Arabia, this report confirms that PFICs, in particular MDR3 deficieny, have a worldwide distribution[14-15] and that are probably underestimated.
CONCLUSIONS

PFIC3 is probably less rare than usually thought. We present a case of Asian origin harbouring novel variant in ABCB4. Morphological studies alone are not sufficient for a firm diagnosis of MDR3 deficiency which requires direct gene testing.

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