

CASE REPORT

Novel Variant *ATP8B1* mutation in a child with progressive familial intrahepatic cholestasis (type 1)

Nida Mirza^{1*}, Ravi Bharadwaj², Smita Malhotra³, Anupam Sibal⁴

ABSTRACT

Background: Progressive familial intrahepatic cholestasis (PFIC) is a group of heterogeneous autosomal recessive disorders attributed to hepatocellular cholestasis, characterized by low serum γ -glutamyl transferase (GGT) levels due to mutation in *ATP8B1*.

Case Presentation: We present a case of 2-year-old male child who experienced persistent marked pruritus, jaundice, and failure to thrive since 3 months of age. He was diagnosed as PFIC on the basis of histology, biochemical, and clinical finding. On genetic analysis by next generation sequencing, a novel homozygous missense variation in exon 19 of the *ATP8B1* gene [chr18:g.55335672C>T; Depth: 71x] resulting in the amino acid substitution of Glutamic acid for Glycine at codon 733 [p.Gly733Glu;ENST00000536015.1], which was confirmed by sanger sequencing of parents.

Conclusion: We report a case of PFIC type 1 with a novel homozygous missense variation in exon 19 of the *ATP8B1* gene with both mother and father as heterozygous carrier. Further confirmation of this variant in *ATP8B1* mutation will occur by identification of similar phenotypes with similar mutation.

Keywords: Liver disease, case report, novel mutation, progressive familial intrahepatic cholestasis.

Introduction

Progressive familial intrahepatic cholestasis (PFIC) is a group of autosomal recessive disorders leading to cholestatic liver diseases that may progress to decompensated liver disease. They mostly present in the neonatal age or in early infancy. With an incidence somewhere between 1:50,000 and 1:100,000, these represent about 9%–12% cases of infantile cholestasis and are a major indication for liver transplantation in children (1). Physiologically elimination of bile components is done through the canalicular membrane that has transport proteins on the canalicular domain. PFIC type 1 is caused by a mutation in *ATP8B1* gene and is characterized by low γ -glutamyl transferase (GGT) cholestasis, recurrent diarrhea, and pruritus. Extra gastrointestinal manifestations include pancreatitis, sensorineural hearing loss, and growth retardation. In this case, we report a 2-year-old male child who experienced persistent marked pruritus, jaundice and failure to thrive since 3 months of age. He was diagnosed as PFIC on the basis of histology, biochemical, and clinical findings. On genetic analysis by next generation sequencing, a novel mutation in *ATP8B1* gene was found.

Case Presentation

A 2-year-old male child, born at term, product of third-degree consanguineous marriage, presented with complaints of severe pruritus, jaundice, growth failure, and recurrent diarrhea since 3 months of age. He was fourth in birth order with no history of any prior sibling death. There was no history of similar illness in any family member. Antenatal period was uneventful without any history of jaundice or pruritus in the mother. There was no history of bleeding, abdominal distension, altered sensorium, or fever. Physical examination showed wasting (weight 6.7

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kg, <1 centile), stunting (height 72 cm, <1 centile), frontal bossing, wide open anterior fontanelle, icterus, scattered scratch marks, and hepatomegaly (liver span of 10 cm). There was no splenomegaly or ascites. Lab investigations revealed conjugated hyperbilirubinemia [total serum bilirubin (TSB) 11.2 mg/dl, direct serum bilirubin (DSB) 8.9 mg/dl], high serum alkaline phosphatase [613 U/l] with normal levels of serum transaminase [alanine transaminase (ALT) 35 U/l aspartate transaminase (AST) 62 U/l], and γ -glutamyltransferase (24 U/l). Synthetic functions of liver were preserved [albumin 4.0mg/dl, INR 0.9]. Ultrasound suggested enlarged liver with normal echotexture. Liver biopsy revealed maintained acinar structure, portal, and periportal fibrosis with occasional thin bridges. Portal tracts had moderate mixed inflammation comprising of neutrophils, lymphocytes, few eosinophils, and moderate ductular reaction. Bile duct loss was noted in a few small tracts. Hepatocytes showed mild to moderate intracytoplasmic and canalicular cholestasis (Figure 1). Immunohistochemistry of biopsy tissue showed nondeficient Bile salt exporter protein and multi drug resistance 3. Exome sequencing was sent to confirm diagnosis which revealed homozygous missense variation in exon 19 of the *ATP8B1* gene [chr18:g.55335672C>T; Depth: 71x] resulting in the amino acid substitution of Glutamic acid for Glycine at codon 733 [p.Gly733Glu;ENST00000536015.1]. In exome sequencing, mutations were annotated using published variants in literature and a set of diseases databases, common variants were filtered based on allele frequency in 1000Genome Phase 3, exome aggregation consortium (v1.0), gnomAD (v2.1), exome variant server, single nucleotide polymorphism database (v151), and internal Indian population database. Non-synonymous variants effect was calculated using multiple algorithms such as PolyPhen-2, sorting intolerant from tolerant (SIFT), MutationTaster2, and likelihood ratio test. As this mutation was not reported earlier, sanger sequencing of parents was done. Sequence chromatogram and alignment to the reference sequence showing the variation in exon 19

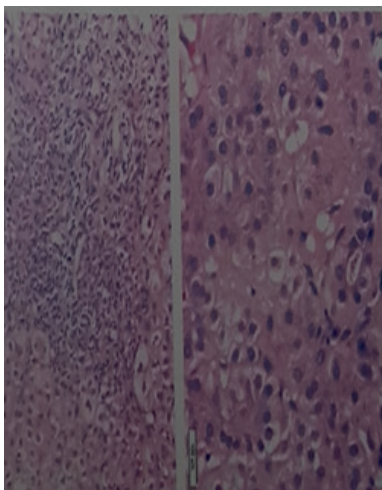


Figure 1. Liver biopsy.

of the *ATP8B1* gene [chr18:g.55335672C>T; c.2198G>A; p.Gly733Glu] was detected in heterozygous condition in the parents, father (Figure 2), and mother (Figure 3). The diagnosis of PFIC type 1 was thus confirmed. On medical management, his pruritus did not show any significant improvement to ursodeoxycholic acid, antihistaminics, cholestyramine, and rifampin. He then underwent an internal biliary diversion procedure for refractory pruritus. Initially, there was significant decrease in pruritus, total serum bilirubin (TSB 2.2, DSB 0.8 after 7 days of procedure); however, the pruritus reappeared again after 4 months of diversion. Lab reports showed rise in bilirubin and serum transaminases (TSB 9 mg/dl, DSB 7.2 mg/dl, AST 110 U/l, ALT 98 U/l, GGT 62 U/l after 180 days of procedure).

Discussion

PFIC type 1 patients typically present in infancy with recurrent intrahepatic cholestasis, pruritus, recurrent diarrheal episodes, fat soluble vitamin deficiencies, and growth failure (1). In this type of PFIC conjugated hyperbilirubinemia with normal or low serum GGT levels is encountered. Bland cholestasis with coarse, granular bile is usually seen in liver biopsy (2) and can provide diagnostic clues using electron microscopy and immunohistochemistry. The confirmation of disease requires genetic testing. Our patient had cholestasis, growth retardation, recurrent diarrhea, severe pruritus since infancy, and intrahepatic cholestasis on liver biopsy which are in favor of PFIC type 1. On genetic analysis, a novel homozygous missense variation was detected in exon 19 of the *ATP8B1* gene [chr18:g.55335672C>T; Depth: 71x] that results in the amino acid substitution of Glutamic acid for Glycine at codon 733 (p.Gly733Glu; ENST00000536015.1). A different missense variation affecting the same codon (*G733R*) has previously been reported as one of the compound heterozygous variants in patients affected with progressive familial intrahepatic cholestasis (3). There is no clear correlation between genetic variant and phenotype as the same mutation has been found to be associated with benign and milder forms like benign recurrent intrahepatic cholestasis 1 (*BRIC1*), transient neonatal cholestasis and intrahepatic cholestasis of pregnancy (3). Although many pathogenic mutations in *ATP8B1* gene are known (3–5), missense mutations are less commonly seen in PFIC type 1 patients than nonsense, frame-shift, and large deletion mutations (3). Familial intrahepatic cholestasis 1 (FIC1) protein is encoded by *ATP8B1* gene on chromosome 18 (6–8). In the canalicular membrane of hepatocytes, FIC1 protein is a component of P-type ATPase, which functions as a flippase between outer and inner plasma membrane of hepatic cells for phosphatidylserine (9). This transport of aminophospholipids in between layers of plasma membrane maintain asymmetric distribution of phospholipids in the membrane bilayer and is considered protective mechanism of hepatocytes from high bile salt concentration in canalicular lumen (10,11). Disruption of

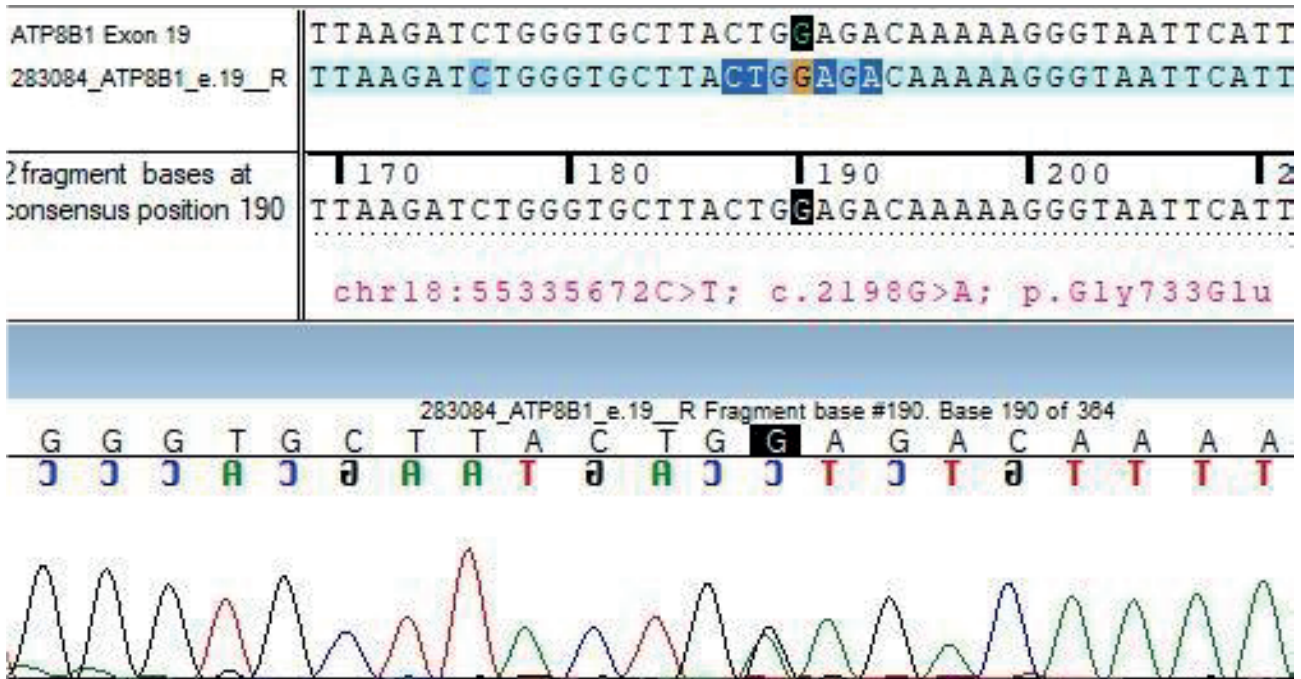


Figure 2. Sequence chromatogram and alignment to the reference sequence showing the variation in exon 19 of the *ATP8B1* gene (chr18:g.55335672C>T; c.2198G>A; p.Gly733Glu) in father.

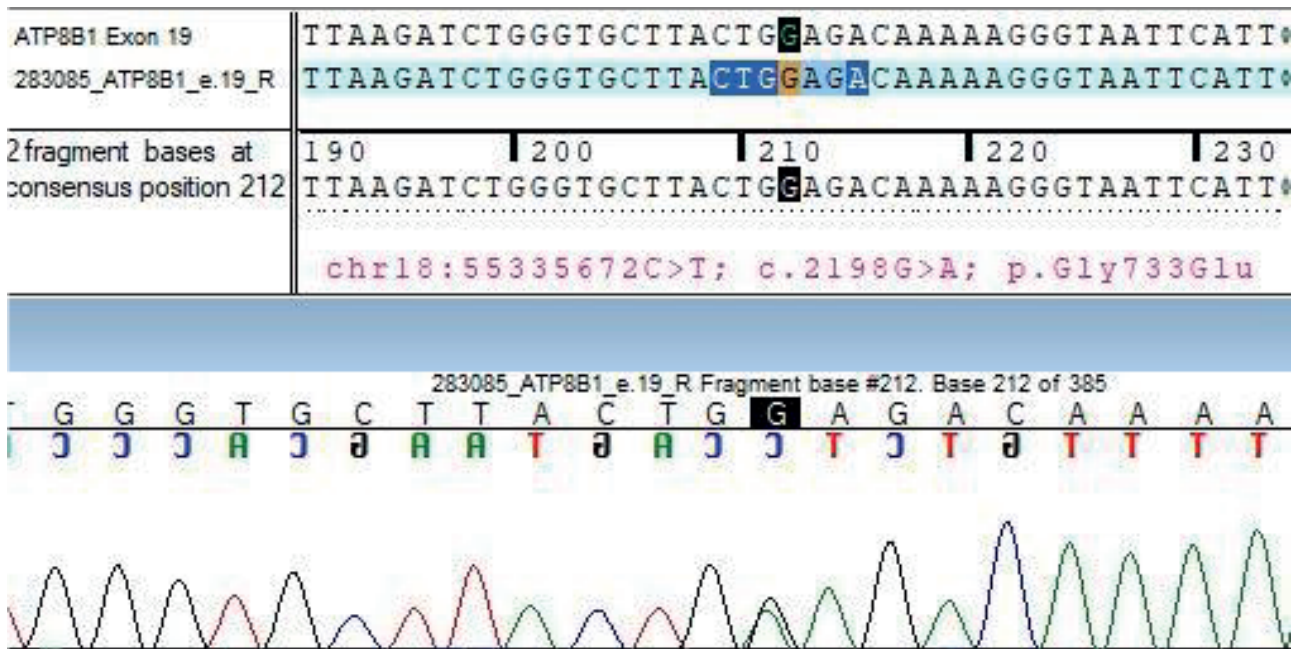


Figure 3. Sequence chromatogram and alignment to the reference sequence showing the variation in exon 19 of the *ATP8B1* gene (chr18:g.55335672C>T; c.2198G>A; p.Gly733Glu) in mother.

this mechanism due to mutation *ATP8B1* gene leads to progressive familial intrahepatic cholestasis.

Conclusion

In conclusion, we report a case of PFIC type 1 with a novel homozygous missense variation in exon 19 of the *ATP8B1* gene with both mother and father as

heterozygous carriers. Further confirmation of this variant in *ATP8B1* mutation will occur by identification of similar phenotypes with similar mutation in other patients.

List of Abbreviations

- ALT Alanine transaminase
- AST Aspartate transaminase

DSB	Direct serum bilirubin
GGT	Gamma glutamyl transferase
MDR	Multi drug resistance
PFIC	Progressive familial intrahepatic cholestasis

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Declaration of conflicting interests

The authors of this article have no affiliations or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval

Ethical approval is not required at our institution to publish an anonymous case report.

Consent for publication

Written informed consent was obtained from the parents.

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