Case Report

Aynur Küçükçongar Yavaş*, Büşra Çavdarlı, Özlem Ünal Uzun, Ayşen Uncuoğlu and Mehmet Gündüz

A novel etiologic factor of highly elevated cholestanol levels: progressive familial intrahepatic cholestasis

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Abstract

Background: Progressive familial intrahepatic cholestasis type 3 (PFIC3) is an uncommon cholestatic liver disease caused by mutations in the ATP binding cassette subfamily B member 4 (ABCB4) gene. Although PFIC3 is frequently identified in childhood, *ABCB4* disease-causing alleles have been described in adults affected by intrahepatic cholestasis of pregnancy, hormone-induced cholestasis, low-phospholipid-associated cholelithiasis syndrome or juvenile cholelithiasis, cholangiocarcinoma and in sporadic forms of primary biliary cirrhosis. Cholestanol is a biomarker which is elevated especially in cerebrotendinous xanthomatosis and rarely in primary biliary cirrhosis (PBC) and Niemann Pick type C.

Case presentation: Here we report a Turkish patient with compound heterozygous mutations in the *ABCB4* gene, who has hepatosplenomegaly, low level of high-density lipoprotein, cholestasis and high level of cholestanol.

Conclusion: This is the first PFIC3 case with a high cholestanol level described in the literature. There are very few diseases linked to increased cholestanol levels, two of which are CTX and PBC. From this case, we can conclude that a high cholestanol level might be another indicator of PFIC type 3.

Keywords: cholestanol; cholestasis; progressive familial intrahepatic cholestasis type 3.

Introduction

Progressive familial intrahepatic cholestasis type 3 (PFIC3) is a rare cholestatic liver disease caused by mutations in the *ABCB4* gene. Mutations in the *ABCB4* gene constitute a heterogeneous group of autosomal recessive disorders that usually present in childhood with the classical phenotype of PFIC. The clinical presentation includes cholestasis, pruritus and jaundice. In contrast to other forms of PFIC, serum gamma-glutamyltransferase (GGT) activity is elevated in ATP binding cassette subfamily B member 4 (ABCB4) disease. This disease is suspected in patients with familial cholestasis, high GGT and unexplained cholestasis [1–3].

There is no information in the literature about cholestanol levels in patients with PFIC3. Cholestanol, which is a cholesterol precursor, can be increased in some diseases including cerebrotendinous xanthomatosis (CTX) and cholestatic liver disease [4].

Here, we report the case of a Turkish patient with compound heterozygous novel mutations in the *ABCB4* gene together with high levels of cholestanol.

Case description

The patient was a Turkish girl born of non-consanguineous parents. She had hepatosplenomegaly, which was determined incidentally during a routine examination when she was 2.5 years old. She also had a doll-like face additionally. The biochemical tests revealed elevated serum liver enzymes, increased GGT, hypertriglyceridemia and low levels of high-density lipoprotein cholesterol (Table 1). Abdominal sonography revealed heterogeneous

***Corresponding author: Assoc. Prof. Aynur Küçükçongar Yavaş,** MD, Pediatric Metabolism, Ministry of Health Ankara City Hospital, University of Health Science, Ankara, Turkey,
E-mail: aynurcon@yahoo.com
Büşra Çavdarlı: Medical Genetics, Ministry of Health, Ankara City Hospital, University of Health Science, Ankara, Turkey
Özlem Ünal Uzun and Mehmet Gündüz: Pediatric Metabolism, Ministry of Health, Ankara City Hospital, University of Health Science, Ankara, Turkey
Ayşen Uncuoğlu: Pediatric Gastroenterology, Sakarya University, Sakarya, Turkey

Table 1: The laboratory values during follow-up.

Hemoglobin, mg/dL	10.1	10	7.6	9.7	9	12.5
White blood cell, /mm^3	5100	5400	3200	3000	3200	7400
Platelet, /mm^3	137,000	167,000	102,000	80,000	102,000	89,000
Aspartate aminotransferase, U/L	126	127	132	252	217	191
Alanine aminotransferase, U/L	89	97	82	179	133	138
Total bilirubin, mg/dL	2.52	2.62	3.34	3.39	311	3.36
Direct bilirubin, mg/dL	1.38	1.28	1.8	1.84	3.39	1.86
γ-glutamyl transferase, U/L	229	238	255	311	177	396
Alkaline phosphatase, U/L	218	176	106	200	290	291
High-density lipoprotein, mg/dL	22	15	12	23	21	22
Total cholesterol, mg/dL	209	254	153	216	198	239
Triglyceride, mg/dL	161	200	225	202	184	180

echogenicity of the liver and hepatosplenomegaly. The patient was evaluated for hepatic glycogenosis, but molecular analysis provided no evidence of glycogen storage diseases. Metabolic screening tests including plasma and urine amino acids, urine organic acids, very long-chain fatty acids and acylcarnitine levels were all within the normal range. The enzymatic activities of glucocerebrosidase, sphingomyelinase and acid lipase were normal. Niemann Pick type C (NPC) disease was excluded by next-generation sequencing of NPC1 and NPC2 exons. Liver biopsy showed bridging fibrosis and chronic cholestasis. The patient did not respond to treatment with ursodeoxycholic acid. The serum cholestanol level was measured two times in different centers. The levels of cholestanol were 33.71 μmol/mL (0.45–3.75) in one center and 114.18 μmol/mL (3–16) in another center. Therefore, a molecular genetic test for CTX disease was performed. No mutations were detected in the *CYP27A1* gene. Because of the chronic cholestasis and high level of GGT, the patient was referred for medical genetics consultation to explore the possibility of PFIC.

Next-generation sequencing of target genes

Informed consent was received from the parents of the patient. *NPC1*, *NPC2*, *CTX*, *CYP27A1* and *PFIC3* gene sequence analyses were performed step by step with next-generation sequencing via the Miseq platform (Illumina, San Diego, CA, USA). Sequences that are aligned to the hg19 genome were analyzed with the Miseq Reporter. Two different and novel mutations were identified on different alleles of the *ABCB4* gene: *NM_018849.2:c.1565>C* and *NM_018849.2:c.3859T>A*. Based on this result, the patient was diagnosed with PFIC3.

Whole exome sequencing

Whole exome sequencing (WES) was performed to determine whether the patient had any other pathogenic variants that could cause a high cholestanol level. For the WES approach, the SureSelect Human All Exon V6 capture kit (Agilent Technologies, Santa Clara, CA, USA) was used together with the Novaseq 6000 platform (Illumina, San Diego, CA, USA) for sequencing. Data were analyzed using the Verita Trekker® Variation Site Detection System and the Enliven® Variation Site Annotation Interpretation System, independently developed and validated by Berry Genomics. The resulting variants were filtered against public databases, including 1000 Genomes phase 3, ExAC database version 0.3 and gnomAD, to retain variants that were found at an allele frequency of less than 5%. Data interpretation rules were set according to the recommendations and guidelines of the American College of Medical Genetics and Genomics (ACMG).

Discussion

Liver diseases of various etiologies have the potential to disrupt hepatic sterol metabolism. Cholestanol is a non-cholesterol sterol metabolite of cholesterol that reflects cholesterol absorption under physiologic conditions. Cholestasis results in elevated serum cholestanol concentrations [5]. Reduced bile acid synthesis may contribute to an increased serum cholestanol level. Studies on primary biliary cirrhosis (PBC) suggest that low serum ratios of lathosterol:cholesterol and campesterol:sitosterol but high ratios of cholestanol:cholesterol reflect advanced PBC [6].

CTX is a rare autosomal recessive disorder of cholesterol and bile acid metabolism that can cause significant systemic and neurologic abnormalities. This disease is

another cause of increased cholestanol levels. It has been documented that a lack of chenodeoxycholic acid (CDCA) production in CTX patients results in the overproduction of cholestanol, which accumulates in the tendons and brains of these patients. Treatment with oral CDCA results in a significant reduction in plasma cholestanol levels, often into the normal range [7].

Progressive familial intrahepatic cholestasis is a heterogeneous group of rare autosomal recessive liver disorders of childhood, characterized by mutations in genes that encode proteins involved in the hepatocellular transport system. Three main subtypes of PFIC (PFIC1, PFIC2 and PFIC3) have been identified. Clinically, intrahepatic cholestasis is the main manifestation of this disease, which is classified into either the low GGT type or high GGT type based on glutamyl transpeptidase activity. Liver biopsy of patients with the high GGT type (PFIC3 type) usually reveals interlobular bile duct hyperplasia, terminal bile duct biliary sludge formation and periportal edema, suggesting that the cause might be obstructive lesions in the bile duct rather than abnormal bile synthesis.

Progressive familial intrahepatic cholestasis type 3 is caused by homozygous or compound heterozygous mutations in the *ABCB4* gene, located on chromosome 7, which encodes multidrug resistance protein 3. This protein transports phospholipids into the canalicular lumen where it neutralizes bile salts and helps to prevent injury to the biliary epithelia and bile canaliculi [8].

Two novel mutations in the *ABCB4* gene were detected in our case. According to the ACMG guidelines for the interpretation of genetic variants [9], the mutation *c.1565T>C* in the *ABCB4* gene of our patient is classified as "likely pathogenic". The evidence for this classification is as follows: (1) this mutation occurs in the ABC transporter 1 domain, which includes 29 pathogenic variants out of 32 classified variants; (2) the mutation was not found in the GenomAD Genomes and Exomes projects [10]; (3) 79 of all 89 variants of the *ABCB4* gene that are classified as "pathogenic" or "likely pathogenic" are missense mutations; and (4) the mutation is evolutionarily conserved, with a potential functional effect identified by conservative predictions of CADD and GERP software, and the mutation was predicted to be harmful by the protein function prediction software Polyphen and LRT. Finally, the REVEL score (averages the scores of all 21 prediction tools when measuring the pathogenicity of a missense variant) of the c.1565T>C variant is 0.883 (pathogenicity limit >0.46) [11].

The other variant (*NM_018849.2:c.3859T*>A) (p.Ter1287Arg) of the *ABCB4* gene was interpreted by the same method and also received a classification of "likely pathogenic". The pathogenicity criteria of this variant are as follows: (1) the mutation was not found in the GenomAD Genomes and Exomes projects [10]; (2) the variant is located within the termination codon and causes "stop loss", meaning protein synthesis will not stop and the new protein would contain approximately 15 additional amino acids compared to the normal protein; and (3) the variant is in trans position to a pathogenic variant. Sanger sequencing studies confirmed that the mutations are on different alleles (compound heterozygous; Figure 1A).

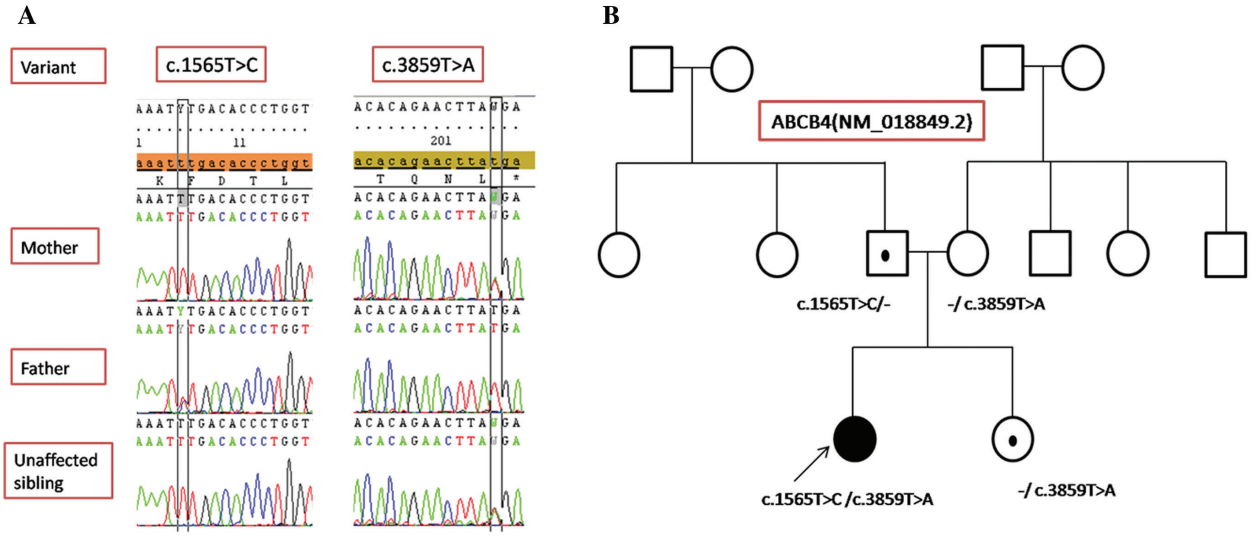

Figure 1: Segregation analysis and pedigree.
(A) Segregation analysis of the family for the mutations on ABCB4 by Sanger sequencing. (B) There was no other affected person in the family, and the sister, father and mother of the patient were diagnosed as carriers of one of the mutations.

Table 2: Quality control parameters of whole exome sequencing and coverage of cholestasis related genes.

Parameters	Values (normal range)
Coverage of target region	97.46%
Fraction of target covered at least 4×	96.96% (>96.7%)
Fraction of target covered at least 20×	94.61%
Average sequencing dept on target	98.23%
Coverage of cholestasis related genes	99.2%
Cholestasis related genes	*ABCB11*, *ABCB4*, *TJP2*, *NR1H4*, *ATP8B1*, *DCDC2*, *CLDN1*, *ABCC2*, *ABCG5*, *ABCG8*, *AKR1D1*, *BAAT*, *CC2D2A*, *CFTR*, *CYP27A1*, *CYP7A1*, *CYP7B1*, *DGUOK*, *DHCR7*, *FAH*, *HNF1B*, *HSD3B7*, *INVS*, *JAG1*, *LIPA*, *MKS1*, *MPV17*, *NOTCH2*, *NPC1*, *NPC2*, *NPHP1*, *NPHP3*, *NPHP4*, *PEX1*, *PEX10*, *PEX11B*, *PEX12*, *PEX13*, *PEX14*, *PEX16*, *PEX19*, *PEX2*, *PEX26*, *PEX3*, *PEX5*, *PEX6*, *PEX7*, *PKHD1*, *POLG*, *SERPINA1*, *SLC25A13*, *SLC27A5*, *SMPD1*, *TMEM216*, *TRMU*, *UGT1A1*, *C14ORF133*, *VPS33B*

WES was performed to investigate whether another mutation that could lead to high levels of cholestanol was present in addition to the *ABCB4* mutations. There were no other pathogenic mutations on genes related to cholestasis or high levels of cholestanol, and the coverage of these genes was about 99.2% (Table 2). WES also confirmed the two different and novel mutations on different alleles of the *ABCB4* gene in our patient: *NM_018849.2:c.1565>C* and *NM_018849.2:c.3859T>A*. According to this result, the patient was diagnosed with PFIC3. There were no other affected persons in the patient's family, but her sister, father and mother were all diagnosed as carriers of one of the mutations (Figure 1B).

There is limited knowledge about the relationship between thyroid-stimulating hormone (TSH) and hepatic bile acid homeostasis. One study [12] showed that TSH inhibits bile acid synthesis. We found a heterozygous thyroid-stimulating hormone receptor (TSHR) variant in our patient. This variant could have been interpreted as "likely pathogenic" if the patient presented any disease-related clinical findings. However, the patient's thyroid function test results were within a normal range. Therefore, the identification of this TSHR variant was considered an incidental analysis result, and we decided to continue treatment based on the clinical findings of the patient.

The patient presented with hepatosplenomegaly, cholestasis and a high GGT level. We had determined a high level of cholestanol during an initial evaluation before the certain diagnosis. Based on the results of the analysis, diseases that cause a high level of cholestanol were excluded in our patient. The molecular test revealed two mutations on the *ABCB4* gene, so she was finally diagnosed with PFIC3. For PFIC3 patients, the decrease in biliary clearance of serum cholestanol can be the reason for elevated cholestanol. In order to determine the main reason behind the high level of cholestanol, and to reveal the possible existence of these two new mutations in the trans position in the *ABCB4* gene, protein function studies should be performed.

Conclusions

We presented the case of a patient with PFIC type 3, who had two novel mutations and a high level of cholestanol. To our knowledge, this is the first PFIC3 case with a high cholestanol level described in the literature. There are very few diseases linked to increased cholestanol levels, two of which are CTX and PBC. From this case, we can conclude that a high cholestanol level might be another indicator of PFIC type 3.

What is new:

- Cholestanol could be a new biomarker in patients with PFIC3.
- Two novel mutations of the *ABCB4* gene were identified.

Author contributions: Aynur Küçükçongar Yavaş wrote the paper, Aynur Küçükçongar Yavaş, Özlem Ünal Uzun and Mehmet Gündüz collected the data of the case, Ayşen Uncuoğlu evaluated the case and organized the molecular test for PFIC type3, and Büşranur Çavdarlı analyzed the genetic test. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
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