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Case Report

Non-syndromic perspective on a unique progressive familial intrahepatic cholestasis variant: *ZFYVE19* mutation

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ABSTRACT

Background. *ZFYVE19* mutation has been recently identified as one of the non-syndromic causes of cholestasis. It is associated with elevated gamma-glutamyl transferase levels and is likely a cause of neonatal-onset and intrahepatic cholestasis.

Case. Here, we report a rare case of *ZFYVE19* defect, confirmed by whole exome sequencing (WES). Our patient, who is currently 4 years old, presented to us at the age of 2 years with elevated levels of serum transaminases and bilirubin. WES revealed a homozygous *ZFYVE19* mutation despite preserved synthetic liver function. This gene has recently been identified in the literature as a cause of non-classical progressive familial intrahepatic cholestasis (OMIM # 619849). Treatment with an appropriate dose of ursodeoxycholic acid resulted in the regression of elevated liver enzymes and itching. The patient's body mass index progressively increased throughout the treatment period. No medication side effects were observed at any point. Currently, the patient remains asymptomatic during follow-up.

Conclusion. We have identified the *ZFYVE19* mutation as a variant that is not accompanied by any other symptoms. However, we have limited knowledge about the progression of the disease and are closely monitoring the patient for potential liver-related issues. Using WES in cases of undiagnosed liver enzyme elevations or cholestasis can help identify new genes and improve our understanding of the underlying pathophysiology.

Key words: cholestasis, progressive familial intrahepatic cholestasis, ursodeoxycholic acid, whole exome sequencing.

Cholestasis is defined as the accumulation of bile products in the liver due to a decrease in the formation of bile in hepatocytes or a decrease in the excretion of bile products into the bile ducts/intestinal lumen. It can be acute, chronic, or recurrent, and is seen in approximately 1 in 2500 live births.[1] It can occur in all age groups, including the neonatal period, and can be caused by either intrahepatic or extrahepatic factors. Progressive familial intrahepatic cholestasis (PFIC) refers to a heterogeneous group of diseases that originate in the hepatocytes and have autosomal recessive inheritance. It is considered one of the causes of intrahepatic cholestasis.[2] The incidence of this group of diseases is estimated to be 1 in 50,000 to 1 in 100,000 live births, and both sexes are affected equally. It is known that the three classical types of PFIC are caused by mutations in hepatocyte transport genes involved in bile acid formation.[3]

In this case report, we present a 4-year-old male patient with a rare genetic form of cholestasis not caused by the classical PFIC genes.

Case description

A male patient presented to our clinic at the age of 2 years with complaints of jaundice and itching. In the patient's medical history,

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it was noted that he was monitored due to prolonged jaundice during the neonatal period. At three months of age, he was diagnosed with cholestatic liver disease based on hepatosplenomegaly, elevated transaminases, and jaundice. The patient's parents had a history of first-degree consanguinity. The patient had been using ursodeoxycholic acid (UDCA) for approximately 2 years, and the family discontinued UDCA usage two months prior to the current visit due to a lack of improvement in symptoms. At the age of two years, the patient's anthropometric measurements were as follows: weight -1.4 SDS, and body mass index -1.3 SDS. On physical examination, the spleen was palpable approximately 2 cm below the costal margin. Other system examinations were unremarkable. The family conducted the Caregiver Impression of Severity (CaGIS) assessment to evaluate the patient's itching.[4] The assessment ranged from 1 to 5, with 1 indicating no itching and 5 indicating very severe itching. The CaGIS score was evaluated as 4. Following the patient's presentation to our clinic, a comprehensive etiological screening for cholestasis was initiated through primary and secondary investigations.[5] At the time of admission, Alanine aminotransferase (ALT) was 258 U/L, Aspartate aminotransferase was 279 U/L, Gamma-glutamyl transferase (GGT) was 254 U/L, total bilirubin was 3.2 mg/dL, direct bilirubin was 1.9 mg/dL (Fig. 1a, 1b), fasting blood sugar was 82 mg/dL, albumin was 4.4 g/dL, international normalization ratio (INR) was 1.1, hemoglobin (HGB) was 12.9 g/dL, platelet count (PLT) was 89,000 cells/μL, and white blood cell count (WBC) was 5,200 cells/μL. Detailed metabolic, endocrinological and infectious tests were negative. Celiac disease antibodies yielded negative results. Serum bile acids could not be assessed due to the patient's two-year history of UDCA usage. Concurrently, 20 mg/kg/day of ursodeoxycholic acid was initiated. Abdominal ultrasound revealed coarse, granular liver parenchyme with irregular contours, and an enlarged spleen measuring 142 mm in vertical length. The parenchymal echo was heterogeneous, and increased echogenicity was noted at the portal hilum. Portal venous doppler ultrasound was normal. Upper gastrointestinal endoscopy was performed to investigate possible complications of liver cirrhosis. Grade III esophageal varices and fundic varices were observed so the endoscopic band ligation was applied for the esophageal ones and Propranolol was initiated at a dose of 1 mg/kg/day. Magnetic resonance cholangiopancreatography was normal. The common bile duct also appeared normal. Next generation sequencing (NGS) evaluation was conducted for the patient with no identified etiology, excluding classical PFIC subtypes, potential congenital metabolic disorders, and structural cholestatic diseases. A liver biopsy revealed moderate inflammation, interpreted as cirrhosis with significant fibrosis (fibrosis stage 6/6). Marked ductal proliferation was also observed at the portoparenchymal border. However, a definitive etiological interpretation could not be made (Fig. 2).

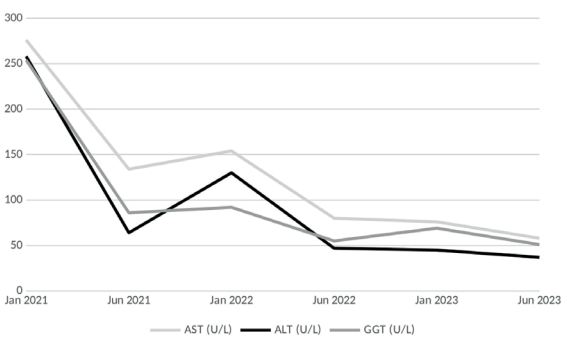

Fig. 1. a. Baseline and follow up values in relation to therapy (ALT: alanine transaminase, AST: aspartate transaminase, GGT: gamma-glutamyl transferase).

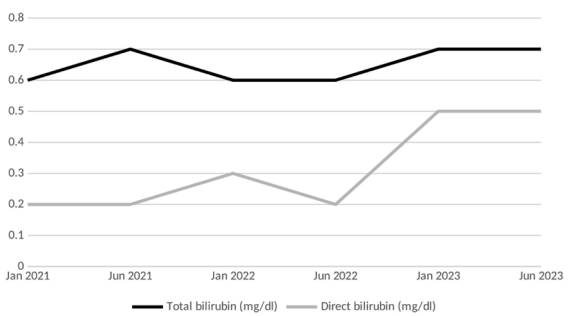

Fig. 1. b. Baseline and follow up values in relation to therapy (Total bilirubin, direct bilirubin).

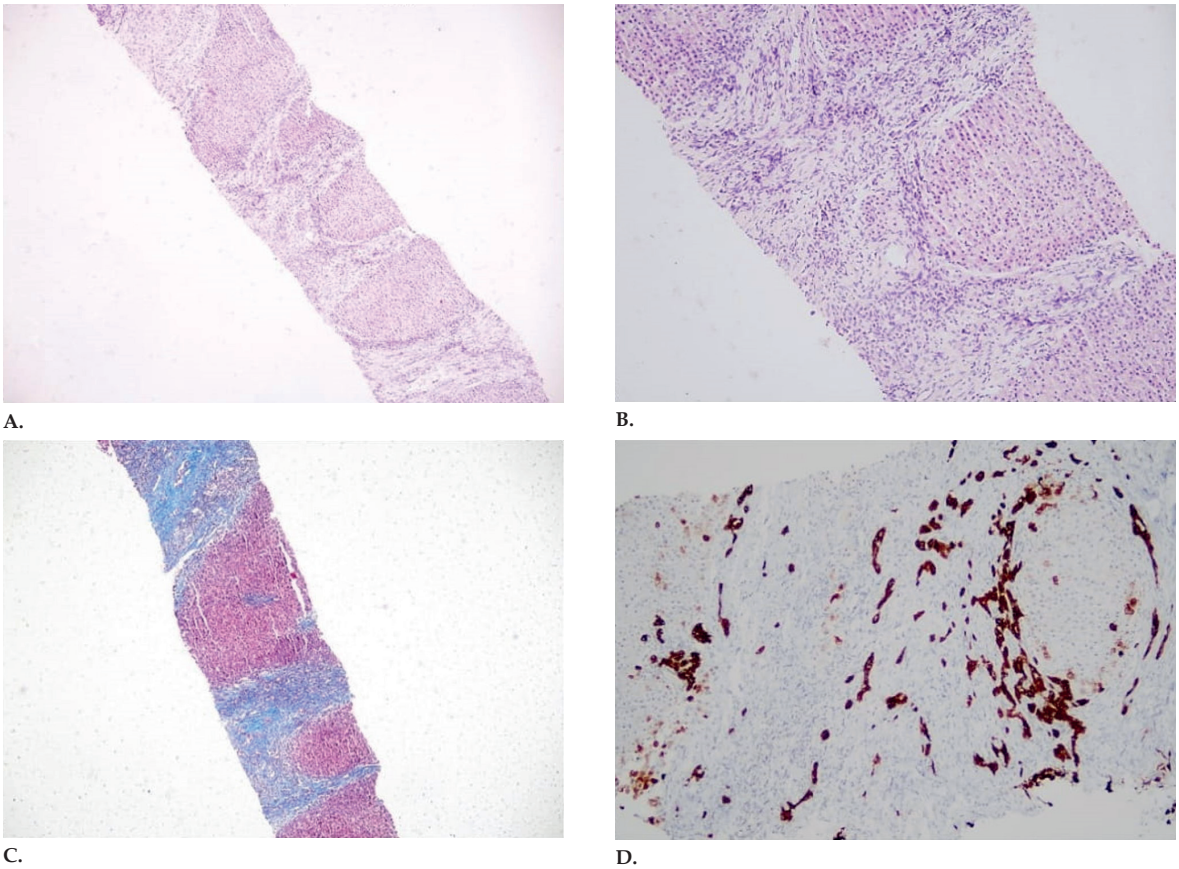

Fig. 2. Liver biopsy pathology sections of the patient. In hematoxylin and eosin sections of the liver biopsy sample, **(A, B)** fibrosis was observed, accompanied by moderate inflammation and proliferation of bile ducts. It is noteworthy that fibrosis is more descriptive in the trichrome stain **(C)**, and it can be interpreted as cirrhosis. **(D)** The immunohistochemical study of CK7 indicates significant proliferation of bile ducts. A: Original microscopic level, B: X100, C: X40, D: X200.

The patient followed up for cryptogenic cirrhosis underwent whole exome sequencing (WES) analysis. A homozygous mutation of c.547C>T (p.Arg183Ter) was identified in the *ZFYVE19* (zinc finger FYVE-type containing 19) gene. This homozygous mutation in the gene led to the diagnosis of a rarely defined genetic cholestasis type (non-syndromic phenotype) known as PFIC9 (OMIM # 619849). The same mutation was detected in both parents as heterozygous. For treatment, the patient was administered ursodeoxycholic acid (20 mg/kg/day), vitamin K (10 mg/week), propranolol (1 mg/kg/day), and fat-soluble vitamin supplementation. Throughout the follow-up period, the patient's clinical and laboratory findings did not deteriorate.

At present, the patient is 4 years old, with normal transaminase levels, serum albumin, and INR. Serum conjugated bilirubin levels were normal. An upper gastrointestinal endoscopy performed for control purposes revealed grade I esophageal varices, with no fundic varices observed. The patient did not experience any gastrointestinal bleeding, and the itching complaint was resolved with current treatments. The family conducted the CaGIS assessment to evaluate the patient's itching for the second time; the CaGIS score was 1. No side effects were observed in the patient while receiving all these treatments. The body mass index increased from -1.3 SDS to -0.49 SDS during the follow-up period. After starting UDCA treatment at an appropriate

dose, liver transaminases and bilirubin values gradually decreased, and the itching complaint completely regressed. Our follow-ups, ongoing for two years, continue without any complaints from the patient. However, hepatosplenomegaly persists. During follow-ups, renal and neurological functions, as well as psychomotor development, were evaluated by specialists and found to be within normal limits. Written informed consent was obtained from the patient's parents for the publication of this case report.

Discussion

PFIC is a genetic disease that can progress to liver cirrhosis due to defective transport of the bile ducts. It can manifest either asymptomatically, such as incidentally detected hypertransaminasemia, or as an end-stage liver disease.[6] In symptomatic cases, treatment options include a few effective drugs, biliary diversion procedures to ensure bile flow or liver transplantation. Medications used for symptom treatment include rifampicin (5 mg/kg/day), UDCA (20-30 mg/kg/day), and odevixibat (40 μg/kg/day).[7] Diagnosis is established through clinical, biochemical, radiological, and histopathological findings, supported by genetic studies. Subtypes are traditionally categorized into PFIC1, PFIC2, PFIC3, PFIC4, and PFIC5, with corresponding genes identified as *ATP8B1, ABCB11, ABCB4, TJP2*, and *NR1H4*, respectively.[6,8] With the increasing focus on genetic studies, new and rare subtypes of PFIC have been identified. These include PFIC6, PFIC7, PFIC8, PFIC10, PFIC11, and PFIC12, with mutations attributed to *SLC51A, USP53, KIF12, MYO5B, SEMA7A*, and *VPS33B*, respectively.

PFIC9 is an autosomal recessive genetic disorder that typically begins in infancy or childhood and is characterized by an increase in serum gamma-glutamyl transferase. Mutations in the *ZFYVE19* gene have been identified as a key regulator of cytokinetic abscission. This gene plays a crucial role in disrupting the bridge between post-mitotic sister cells, leading to the development of the condition.[9] Jaundice, hepatosplenomegaly, portal hypertension, or upper gastrointestinal bleeding may manifest clinically. Patients often benefit from UDCA therapy, with liver enzymes occasionally remaining within the normal range. However, some patients may still require liver transplantation despite treatment. Liver biopsies may reveal micronodular cirrhosis, portal dilation accompanied by fibrosis, bile duct proliferation, and ductal plate malformation.[10] Significant bile duct proliferation was observed in our case, and there was a noticeable moderate lymphocyte-rich inflammation in the portal areas. Liver histopathology revealed a cirrhotic process with marked fibrosis.

Pepe et al.[11] described the case of a 6-year old female patient with a homozygous *ZFYVE19* mutation, previously treated with rifampicin and UDCA but still experiencing resistant itching. They successfully tried odevixibat as an alternative. In our case, we started our treatment with UDCA as medical treatment and we are since continuing.

The choice of medical treatment should be evaluated based on the patient's clinical presentation. However, how long patients can continue using their liver with the selected medical treatment, or if it is possible to preserve the patient's liver, is a question that remains unanswered.

In this diagnosis, we anticipate that the frequency of the "idiopathic neonatal hepatitis" diagnosis will decrease with the assistance of emerging next-generation techniques.[12] PFIC9, recently identified as a new cause of hypertransaminasemia, was diagnosed in our patient through WES analysis. Following cytokinetic abscission, midbodies move toward the cell surface and participate in ciliogenesis. Therefore, any intervention in ZYFVE19 expression can lead to abnormal chromosome segregation and DNA damage.[9] It has been reported that individuals with *ZFYVE19* mutations may also experience cilia dysfunction. Molecular results even indicate that cilia dysfunction is observed in

unaffected cells in individuals with *ZFYVE19* mutations, suggesting a broader impact beyond cholestasis.[13] Unfortunately, due to the unsuitability of laboratory conditions, we were unable to assess investigations into cilia dysfunction in our patient. As new-generation techniques continue to aid in the understanding of genetic disorders, we believe that the identification of specific genetic causes, such as PFIC9, will contribute to more accurate and targeted diagnoses, reducing the prevalence of previously labeled idiopathic conditions like idiopathic neonatal hepatitis.

The important point to consider here is that the *ZFYVE19* mutation is the cause of a newly defined disease called PFIC9, which is also one of the causes of idiopathic neonatal hepatitis to date. It is important to continue to monitor these patients because we do not yet know how much and how it will affect the liver, how and when it will affect other organs that are not phenotypically affected, or whether it will involve other systems. In addition, In addition, WES analysis increases the diagnosis rate in cases of cholestasis with unknown cause, it should be used more widely. Even if it is not applied routinely, it will increase current knowledge and, as a result, studies for the treatment of the newly defined genes.

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Ethical approval

Written informed consent was obtained from the patient's parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CFO, NB; data collection: CFO, EGB, MA, YME; analysis and interpretation of results: CFO, OB, MA, NB; draft manuscript preparation: CFO, MA, EGB, MA, YME. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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