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Progressive Familial Intrahepatic Cholestasis Type 3 – Overlapping presentation with Wilson disease

Ramya Ramraj, MD¹, Milton J Finegold, MD², and Saul J. Karpen, MD, PhD¹

¹Texas Children's Liver Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030, USA

²Department of Pathology, Baylor College of Medicine, Houston TX

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Introduction

PFIC3 (Progressive Familial Intrahepatic Cholestasis type 3) is an inherited cholestatic disorder caused by mutations in the *ABCB4* gene encoding the Multidrug Resistance Protein 3 (MDR3) protein.¹ PFIC3 typically presents during infancy or early childhood, often progressing to chronic liver disease and cirrhosis, requiring liver transplantation.² Since the clinical features of PFIC3 overlap with many other forms of liver disease in childhood, definitive diagnosis may be problematic or delayed. Here we report two patients, ultimately diagnosed with PFIC3, who initially presented with liver histological features including marked hepatic copper accretion that were considered indicative of Wilson Disease (WD).

Case reports

Patient A first presented at age 11 with elevated liver enzymes and abdominal distension. She had hepatosplenomegaly and moderate ascites. Her past medical history was significant for prolonged neonatal jaundice and gallstones requiring cholecystectomy at age nine. In addition, her mother reported pruritus during the late stages of pregnancy. A liver biopsy revealed hepatocyte ballooning degeneration, chronic inflammation in periportal areas, micronodular cirrhosis and a markedly elevated hepatic copper content (860µg/g). By report she had elevated 24 hour urinary copper with normal ceruloplasmin levels. She was diagnosed with WD and started on chelation therapy with trientine that, by report, led to some improvement in serum liver indices.

At age 13 she was referred to our institution for further management and evaluation for liver transplantation. She appeared healthy, was 19th percentile for height & 24th percentile for

Corresponding Author: Saul J. Karpen, MD PhD, Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine and Texas Children's Hospital, 6621 Fannin Street, MC CC1010.00, Houston, Texas 77030, Tel: (832) 822 3616, Fax: (832) 825 3633, skarpen@emory.edu.

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Ramraj: study concept; data acquisition and analysis; drafting and revision of the manuscript Finegold: histological evaluation; manuscript preparation and revision Karpen: study concept & design; data analysis; revision of the manuscript

weight with moderate hepatosplenomegaly. Serum liver indices were AST 85 u/l, ALT 80u/l, Alkaline Phosphatase 774 u/l, GGT 293 u/l, Albumin 2.6gm/dl, Conjugated bilirubin 0.0 mg/dl. The ceruloplasmin level was 26.7 mg/dl (ref 18–46), serum copper 84 µg/dl (ref 90–190), and urine 24 hour copper 125 µg (ref 3–50). At that time she was on chelation therapy with trientine. A liver biopsy (Fig-Panels A, B, C) revealed inflammation, lobular disarray and cirrhosis with detectable copper deposition in periportal hepatocytes (hepatic copper content 191µg/g). Full exon sequencing of the *ATP7B* gene (WD gene) did not reveal any mutations. She was then evaluated for other causes of cholestatic disorders. *ABCB4* gene sequencing was recently commercially available, and analysis revealed compound heterozygosity with known deleterious mutations in the *ABCB4* gene (see Table), leading to the diagnosis of PFIC3. After diagnosis, chelation was discontinued and Ursodeoxycholic acid (UDCA) therapy at 20 mg/kg/d initiated. Liver indices remained stable over the next four years.

Patient B was referred by his pediatrician at the age of six for jaundice, elevated serum liver indices and dark urine. His past medical history was unremarkable and physical examination revealed minimal scleral icterus and hepatosplenomegaly. Serum liver indices were AST 192 u/l, ALT 139 u/l, Alkaline Phosphatase 397 u/l, GGT 608 u/l, Albumin 4.5 gm/dl, Conjugated bilirubin 0.6 mg/dl. Twenty-four hour urinary copper excretion was elevated (66 µg/day) and ceruloplasmin level was normal at 44.6 mg/dl. A liver biopsy (Fig-Panels D, E, F) revealed hepatic inflammation, bridging fibrosis, histochemically detectable copper and elevated total hepatic copper (863 µg/gm), suggesting the potential diagnosis of WD. While awaiting results of the *ATP7B* and *ABCB4* gene sequencing, the patient was started on chelation therapy. Full sequence analysis of the *ATP7B* gene did not reveal any mutations. However, *ABCB4* gene sequencing confirmed the diagnosis of PFIC3, due to the presence of deleterious mutations (see Table), leading to discontinuation of chelation and initiation of UDCA therapy at 20 mg/kg/d. His liver indices continue to be stable for the past 18 months.

Discussion

Determining the underlying etiology of cirrhosis in childhood is sometimes problematic and a relatively recent addition to our diagnostic toolkit includes sequencing of genes which, if mutated, contribute to the development of many chronic liver diseases. These two patients, with marked hepatic copper overload and liver histology consistent with WD have defects in a hepatobiliary transporter gene for phospholipids (*ABCB4*) and not the gene responsible for WD (*ATP7B*). Moreover, this diagnosis underscores the advantages of incorporating specific genetic testing for diagnosis and that such utilization can potentially change treatment options.

PFIC3 results from either monallelic or biallelic mutations in the *ABCB4* gene located on Chromosome 7 encoding for the Class 3 Multidrug Resistance Protein (MDR3).¹ This gene product is predominantly expressed in the hepatocellular canalicular membrane and is responsible for phospholipid transport into bile. Decreased or absent function of the MDR3 transporter results in impaired phospholipid secretion, previously identified as “low phospholipid syndrome”.³ Injury to the biliary epithelium occurs from continuous exposure to hydrophobic bile salts, whose detergent effects are not attenuated by phospholipids. Further, the altered ratio of phospholipids and bile salts leads to increased lithogenicity of bile, resulting in crystallization of cholesterol with cholelithiasis. Injury to the biliary epithelium results in cholestasis, biliary cirrhosis and hepatocellular failure.

Patients with PFIC3 that manifest early with severe disease tend to have homozygous or compound heterozygote mutations affecting protein function.² These patients often have complete absence of the protein on immunostaining or impaired function of the MDR3

transporter. Patients with either missense or nonsense heterozygous mutations may present with less severe phenotypes,² the most common of them being cholelithiasis in adulthood, or maternal pruritus during pregnancy, as in patient A. However such genotype–phenotype correlations are not precise and are not completely reliable.

Children with PFIC3 often present in the first year of life with clinical signs of cholestasis that leads to progressive liver disease and cirrhosis. Older children and adolescents may have features of portal hypertension, including hematemesis and splenomegaly. PFIC3 patients have elevated serum gamma glutamyl transferase activity (GGT) that differentiates them from those with PFIC 1 and 2. They also show elevations in liver transaminases (AST, ALT), alkaline phosphatase activity, and moderate elevation in bile acids. Ultrasonography of the liver and gall bladder may rarely show biliary stones. Liver histology shows hepatocellular damage, inflammatory infiltration of the portal tract and fibrosis. In advanced disease, extensive fibrosis and biliary cirrhosis are seen. Immunostaining of the MDR3 protein can be variable, ranging from complete absence to diminished staining, depending on the mutation.⁴ The diagnosis of PFIC3 is confirmed by molecular genetic analysis of the *ABCB4* gene.

The two PFIC3 patients reported here presented with cholestasis and significant copper accretion in liver. Impaired copper secretion and copper accumulation can be seen in all chronic cholestatic disorders; however the quantity of copper was significant to meet diagnostic criteria for WD. WD is caused by mutations in the *ATP7B* gene that encodes for the copper transporting P1 type ATPase.^{5,6} In hepatocytes, this protein is required for proper copper excretion into bile. In the two patients, MDR3 deficiency led to altered copper excretion, but the mechanism remains to be elucidated. Moreover, this diagnosis altered therapy from copper chelation to UDCA, since to date, there are no known beneficial effects of chelation in PFIC3, or other non-WD or cholestatic conditions. Thus, the two cases underscore the importance of molecular genetic testing in the accurate diagnosis of cholestatic disorders that directly affects management decisions and patient outcomes.

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Abbreviations

MDR3	multidrug resistance protein 3
PFIC3	progressive familial intrahepatic cholestasis 3
UDCA	ursodeoxycholic acid
WD	Wilson Disease

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Figure.

Patient A, liver biopsy at age 14 (A–C): [A] micronodular cirrhosis (Masson trichrome x50); [B] bile duct proliferation without bile retention (arrows) (hematoxylin-eosin x100); [C] hepatocellular copper (Rhodanine stain × 200)

Patient B, liver biopsy at age 6 (D–F): [D] micronodular cirrhosis with severe chronic active inflammation (Masson trichrome x62); [E] portal and lobular inflammation; two small bile ducts (arrows) one with bile plug (hematoxylin-eosin x250); [F] hepatocellular copper (Rhodanine stain x200)

Table 1

Patient	A	B
Age (Y)	13	5
AST (U/L)	85	187
ALT (U/L)	80	132
Alk Phosphatase (U/L)	774	346
GGT (U/L)	293	512
Albumin (G/DL)	2.6	4.4
Direct Bilirubin (MG/DL)	0.0	0.4
Ceruloplasmin (MG/DL)	26.7	44.6
<i>ABCB4</i> gene mutation	c.2563C>T(p.Q855X) c1283T>C(p.V428A)	c.490T>G(p.W164G) c.3081+1 G>C
Hepatic Cu Content (µg/gm)	860	863