Unusual Cystic Fibrosis Transmembrane Conductance Regulator Mutations and Liver Disease: A Case Series and Review of the Literature

H.H. Khan[a], N.A. Mew[b], S.S. Kaufman[a], N.A. Yazigi[a], T.M. Fishbein[a], and K.M. Khan[a,*]

[a]Transplant Institute, Medstar Georgetown University Hospital,Washington, DC, USA; and[b]Medical Genetics, Children's National Medical Center, Washington, DC, USA

ABSTRACT

Cystic fibrosis (CF) is caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene, deranging the activity of chloride channels on the epithelial cell surface. Herein we describe end-stage liver disease in 3 infants with rare CFTR gene mutations; 2 of them were heterozygous. Case 1 was a premature male infant with negative CF screening at birth who developed a small bowel obstruction in the neonatal period requiring an ileostomy, with subsequent cholestatic liver disease and portal hypertension. In addition, he was noted to have frequent respiratory infections prompting a sweat test, which was positive. Genetic testing revealed that he was heterozygous for P.1177F. He then underwent a successful liver transplant. Case 2 was a female infant who developed progressive cholestasis with poor weight gain and was found to have neonatal hepatitis on liver biopsy. A sweat test was negative and genetic testing revealed she was heterozygous for CFTR and PEX26 gene mutations. She subsequently developed pneumatosis involving the cecum that was treated conservatively, followed by a successful liver transplant. Case 3 was a male infant who developed progressive liver disease, with liver biopsy showing neonatal hepatitis. He was extensively investigated but had a negative sweat test on repeated studies. Genetic testing revealed that the patient was heterozygous P.K186N-variant in the AKRID1 gene and homozygous P.R75Q-variant in the CFTR gene. Unfortunately, he succumbed to an acute upper gastrointestinal hemorrhage. Rare and unusual CFTR mutations, even in the heterozygous form, may be a feature in otherwise undiagnosed end-stage liver disease of infancy.

THE CENTRAL role of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in the pathophysiology of cystic fibrosis (CF) is well established. In the hepatobiliary system, the CFTR gene is expressed only in the epithelium of bile ducts and gallbladder and not in hepatocytes or other hepatic cells. As a result, the pathologic effects in the hepatobiliary system are related to biliary clearance [1]. The prevalence of CF-associated liver disease is 2%–37% in children and young adults [2]. Approximately 5%–10% of patients with CF develop cirrhosis during the first decade of life [3]. This is determined by genetic mutations, nutritional status of the patient, and iatrogenic causes [4]. Neonatal cholestasis is a known, yet uncommon presentation of CF, occurring in <5% of affected patients [5]. Although 1900 mutations have been identified, their role in neonatal cholestasis has not been defined [6].

We describe 3 cases of rare CFTR gene mutations in infants who did not have a clinical diagnosis of CF. Nevertheless, all 3 patients required liver transplantation. We propose that uncommon CFTR variants may be consequential in cholestatic liver disease that can progress to liver failure in early childhood.

*Address correspondence to Khalid M. Khan, MD, Transplant Institute, Medstar Georgetown University Hospital, 3800 Reservoir Road NW, Pasquerilla Healthcare Center, 2nd Floor, Washington, DC 20007. E-mail: khalid.m.khan@gunet.georgetown.edu

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CASE REPORTS

Case 1

A premature male infant born at 26 weeks gestation had failed feeding trials, and a barium enema on day 26 of life revealed a microcolon and meconium ileus. The newborn immune-reactive trypsinogen screen for CF was negative and a rectal biopsy on day 52 was negative for Hirschsprung disease. On day 63 of life, the patient underwent an exploratory laparoscopy with ileostomy placement 2 cm from the ileocecal valve; the patient was noted to have a persistently elevated bilirubin at this time, although liver ultrasound was normal and without evidence of portal hypertension. He had also received parenteral nutrition intermittently. The patient became progressively cholestatic with a mark transaminitis and eventually developed ascites that required serial peritoneal taps. A liver biopsy on day 89 revealed neonatal cholestatic hepatitis. A hepatobiliary iminodiacetic acid scan showed normal excretion. A peritoneal-venous (Lavean) shunt was placed. On day 141, he was treated for frank bleeding from the stoma site and a repeat ultrasound, on day 158, showed reversal of portal venous flow.

Throughout this time he remained ventilated; it was noted that he had several pulmonary infections including 2 episodes of respiratory syncytial virus with numerous periods of pulmonary decompensation and an increased ventilator requirement. He therefore underwent a sweat chloride test, which showed a value of 40 mmol/L on the right arm and 39 mmol/L on the left arm, indicating "possible CF." Genetic sequence and deletion/duplication analysis of CFTR revealed heterozygous p.I177F variant in the CFTR gene, and no other mutations, variants of unknown significance, deletions, or duplications were detected. He was started on regimen of pulmozyme, hypertonic saline, and nebulized β_2-agonist treatments and chest physiotherapy.

He was transferred to our transplant center on day 173 of life, and on day 193 he was evaluated for additional causes of cholestatic liver disease of infancy that included α_1-antitrypsin deficiency, genetic disorders of bile excretion, and metabolic and infective causes. He received a cadaveric liver transplant with no major complications in the posttransplant period. The patient's gamma-glutamyltransferase (GGT) at the time of transplantation was 19 U/L, although there was no indication of familial intrahepatic cholestasis based on genetic testing. The explanted liver pathology revealed micronodular cirrhosis associated with marked bile duct proliferation, and there was no indication of extrahepatic biliary disease.

Case 2

An African American female infant born at 37 weeks of gestation, with the mother having had gestational diabetes and hypertension, developed jaundice when on breast milk, which improved during the subsequent weeks. However, her stools then turned pale for which she underwent a percutaneous liver biopsy and subsequently an operative cholangiogram. The biopsy revealed neonatal hepatitis and the cholangiogram showed a small but patent bile duct. On genetic testing using a cholestasis panel (EGL genetics, Tucker, GA), the patient was found to be heterozygous for the c.1210-12T [5] reduced function variant (known as the 5T allele) in the CFTR gene and c.200A>G (p.N67S) variant in the PEX26 gene. Further testing included a negative sweat test with sweat chloride values of 10 mmol/L on both the right and left arms.

She became progressively cholestatic with failure to gain weight and difficult-to-control ascites and abdominal distention, necessitating in-hospital care. On day 261 of life, Doppler ultrasound of the liver revealed an inhomogeneous liver texture with reversed portal venous flow and high resistive indices in the hepatic arteries. Further imaging with computed tomography showed extensive pneumatosis involving the ascending, hepatic flexure, and transverse colon (Fig 1). Shortly thereafter, on day 285 of life, she underwent liver transplantation with a Roux-en-Y hepaticojejunostomy after evaluation for additional causes for cholestatic liver disease of infancy that included α_1-antitrypsin deficiency, genetic disorders of bile excretion, and metabolic and infective causes. The patient's GGT 2 days before transplantation was 390 U/L and 1 day after transplantation it was <3 U/L. The histology of the explanted liver revealed micronodular cirrhosis associated with marked cholestasis, peripheral bile duct proliferation, and paucity of bile ducts of the larger portal triads. There was no indication of extrahepatic biliary disease.

She required significant nutritional support with nasogastric feeding early after transplant; stool elastase assessment proved normal and no respiratory symptoms were noted on follow-up.

Case 3

A premature male twin born at 33 week gestation following a normal birth, but with idiopathic neonatal cholestasis since birth was referred for transplant evaluation at around 7 months of age with progressive liver disease accompanied by histologic paucity of the interlobular bile ducts. His Pediatric End-Stage Liver Disease (PELD) score was 35 and clinical findings included scleral icterus, abdominal distention, and breathing difficulty.

The patient had polydactyly of the left hand and plagiocephaly that was not present in the other twin. He underwent an extensive genetics evaluation, including genome sequencing studies. The patient was listed for transplantation after evaluation for additional causes for cholestatic liver disease of infancy that included α_1-antitrypsin deficiency, genetic disorders of bile excretion, and metabolic and infective causes. Additional negative testing included a sweat test on 2 occasions; neonatal and adult cholestasis deletion/duplication panel revealed that the patient was heterozygous c.558A>C (p.K186N)−variant in the AKRID1 gene and homozygous c.224G>A (p.R75Q)−variant in the CFTR gene.

Imaging of the liver showed thrombosis of the portal vein though an ultrasound at 1 month of age with normal portal vein flow. He remained on the cadaveric waitlist for liver transplant for 163 days. Unfortunately, he developed acute gastrointestinal hemorrhage and could not be resuscitated. The patient's GGT remained high throughout this period with a final value of 349 U/L 6 days before his death.

The postmortem showed significant gastrointestinal bleeding with blood clots in the stomach and the entire small bowel. Congestion

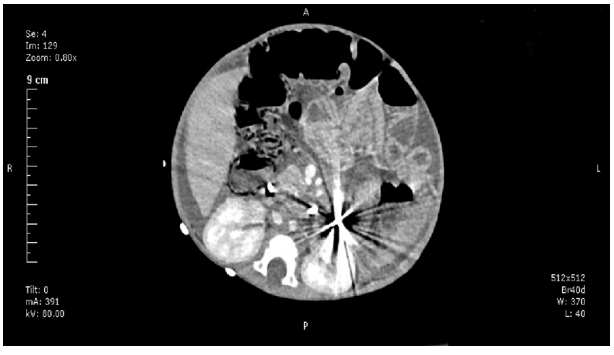

Fig 1. Pneumatosis intestinalis at the level of the hepatic flexure and transverse colon.

was seen in both lower lobes of the lungs, but the parenchyma was well-aerated and pink. The liver was cirrhotic and enlarged with significant nodularity but no evidence of congenital biliary disease.

DISCUSSION

It is well known that the most common CFTR mutation in patients with CF is the deletion of phenylalanine at the 508 position [7]. At least 1 allele is present in 95% of CF patients, and 65%–70% of CF patients are homozygous for this mutation [8].

There are 6 classes of CF mutations [6]. In class 1 there is premature mRNA termination secondary to nonsense or frameshift mutation, resulting in the CFTR protein being completely absent [9–11]. The class 2 mutation has a defect in protein processing due to abnormal posttranslational modification [9]. Protein regulation is defective in class 3, resulting in abnormalities of the nuclear binding fold regions and causing decreased protein activity in response to adenosine triphosphate [9]. Ion flow frequency and duration of channel opening is reduced in class 4 due to a protein conduction defect [9]. In class 5, there is a reduction in the amount of functional CFTR leading to compromise of mRNA and/or mature protein stability [12,13]. In class 6, C-terminus abnormalities lead to enhanced turnover of functional CFTR [9]. The classification cannot be used for accurately predicting the clinical outcomes because there is poor correlation with the clinical phenotype [14]. Nevertheless, general trends have been noted; the mutations of classes 1–3 are very severe due to minimal function of the CFTR protein and thus are associated with severe phenotypes, including pancreatic insufficiency, meconium ileus, CF-associated diabetes, and CF-associated liver disease [15–18]. On the other hand, class 4 and 5 mutations are associated with less severe clinical outcomes [18].

There are limited data on CF-related cholestasis. CF can mimic biliary atresia presenting with acholic stools [19]. Eminoglu et al reported the case of a 63-day-old male infant who had a homozygous, nonsense c.3871 G>T (p.G1247X) mutation in the CFTR gene [20]. Liver biopsy revealed canalicular cholestasis and extensive vacuolar degeneration in the hepatocytes without appreciable inflammation and bile duct proliferation [20]. The child was treated successfully with ursodeoxycholic acid and oral pancreatic enzyme replacement [20]. A similar case was reported by Harris et al, where a premature male newborn was positive for R117H mutation and 5T variant on separate chromosomes of the CFTR gene [21]. The patient's hyperbilirubinemia resolved on its own.

The p.I177F variant of CFTR gene (case 1) has not been reported as causing neonatal cholestasis in homozygous or heterozygous form as far as we can determine. Ko et al reported a 4-month-old male child who had double heterozygous nonsense mutations, c.263T>G in exon 3 and c.2089_2090insA in exon 13, in the CFTR gene [22]. Similar to our case, the child had meconium ileus at birth in addition to respiratory infections, failure to thrive, fatty liver with hepatomegaly, and cholestasis [22]. The patient required ventilation, antibiotic, mucolytics, and physiotherapy, and was reported to be doing well at the age of 19 months [22].

The c.1210-12T [5] variant (case 2), commonly known as the 5T allele, has been reported as a variant in the general population [23]. The 5T variant in intron 8 can reduce the amount of functional protein produced from normal or mild CF genes altering splicing [23]. This variant has been associated with congenital bilateral absence of the vas deferens (CBAVD) in males [23]. Furthermore, the 5T variant on 1 chromosome, in combination with a known CF pathogenic variant on the opposite chromosome, such as F508, may lead to mild or atypical clinical features of CF [24]. The 5T variant is considered to be one with reduced penetrance [24].

The p.R75Q variant of CFTR is generally regarded as a benign variant [25]. Schneider et al reported that the p.R75Q variant of CFTR in the presence of the SPINK1 mutation was associated with increased risk of chronic pancreatitis [26]. Our patient (case 3) was found to be homozygous for this mutation and developed cholestasis and hepatic cirrhosis. Biallelic mutations have been associated with cholestasis and bile duct paucity and therefore the possible pathogenicity of genetic variants such as this cannot be dismissed. The challenge in this case is how to explain the discordant phenotype of this patient and his confirmed monoamniotic identical twin, who presumably has the exact same genotype, with both exposed to the same intrauterine environment. In addition, whether these mutations are further influenced by other genetic findings, as in our case 2, and whether they combine to result in clinical liver disease is an intriguing area of study.

Our cases have demonstrated that a rare mutation in the CFTR gene, even in heterozygous form, can be associated with life-threatening hepatobiliary disease to the extent of requiring liver transplantation and that the liver disease progresses much more quickly in these cases. As in our case 2 it may be argued that these subtle mutations may not result in the respiratory phenotype that is characteristic of CF. An alternative prospect would be that, as the carrier rate of CFTR mutations in most populations is 1 in 25, there is a chance that we came across 2 subjects heterozygous for a CFTR mutation. However, this would not explain the patient with homozygous CFTR alterations albeit with a rare mutation. We suggest that all infants without a defined cause for cholestatic liver disease undergo an extensive work-up, as delineated by Figiel et al [27]. Also, testing should be done for these rare mutations of the CFTR gene in infants presenting with cholestatic liver disease if a thorough screen for known liver diseases is negative. Exactly how these genetic findings result in liver failure in some cases needs further study but may only come to light with further observation.

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