ABCB4 Disease Presenting with Cirrhosis and Copper Overload—Potential Confusion with Wilson Disease

Benjamin L Shneider

Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA

A 2-year-old female with cirrhosis was found to have a liver copper of 248 µg/g dry weight. She was eventually diagnosed with ABCB4 disease on the basis of heterozygote A546D and R176W mutations. Her liver disease was partially responsive to ursodeoxycholic acid therapy. Copper overload occurs in cholestatic liver disease and this must be considered to avoid misdiagnosis of Wilson disease. (J CLIN EXP HEPATOL 2011;1:115–117)

The last decade has seen a remarkable growth in both the identification of specific etiologies for the range of chronic liver disease in children and in the new disease-specific therapeutic regimens. These developments not only provide new optimism for the prognosis for children with chronic liver disease, but also place a greater burden on clinicians to make accurate diagnoses. Careful analyzes of existing diagnostic paradigms are critical to ensure that the paradigms are accurately employed. In addition, a plethora of molecular diagnostic techniques have been developed, although interpretation of their results is not always straightforward.

The prevalence and treatability of Wilson disease makes it a very important diagnosis to consider in all children with chronic liver disease. The prevalence of Wilson disease has been estimated to be 30 per million individuals.¹ Thus, though not common, Wilson disease is also not rare. The clinical presentation of Wilson disease can be quite protean, making its consideration paramount in the assessment of most children with chronic liver disease. If diagnosed in a timely fashion, Wilson disease is remarkably responsive to pharmacologic therapy, but misdiagnosis can lead to irreversible complications and possible death. Unfortunately, diagnostic paradigms for Wilson disease are complex and not completely sensitive or specific.^{2,3} We present a case of a child who was initially considered to have Wilson disease, and was ultimately diagnosed with liver disease secondary to ABCB4 deficiency (MDR3 disease or progressive familial intrahepatic cholestasis type 3).

Accurate diagnosis permitted institution of appropriate disease-specific therapy.

CASE REPORT

A two-year-old female patient was initially assessed for abdominal distention and failure to thrive. Initial screening investigations did not reveal evidence of hepatitis B or C, autoimmune hepatitis, celiac disease, alpha-1-antitrypsin deficiency or cystic fibrosis. As a part of her diagnostic work-up, a liver biopsy was performed, the analysis of which revealed a copper level of 248 µg/g dry weight of liver. Serum ceruloplasmin was 28 mg/dL, while serum copper was 1013 µg/L. A diagnosis of Wilson disease was entertained and the child was referred to our program for consultation.

At the time of initial consultation she was noted to be asymptomatic. There was no history of bleeding or pruritus. She was active and did not have any significant fatigue. Her development had been normal to that time. Her family history was unremarkable. On physical examination, she appeared somewhat malnourished with diminished subcutaneous fat and muscle mass. Her weight and height were at the 3rd percentile, despite a midparental height at the 50th to 75th percentile (Figure 1). Transcutaneous oxygen saturation was 98% in an upright position in room air. Her liver was firm and palpable 4 cm below the costal margin. Her spleen was palpable 9 cm below the costal margin. She did not have ascites. Screening laboratory studies were notable for biochemical evidence of liver disease (alanine aminotransferase [ALT] 178 IU/L [normal <40], aspartate aminotransferase [AST] 402 IU/L [normal <40], gamma-glutamyl transpeptidase [GGT] 405 IU/L [normal <50]), intact hepatic synthetic function (albumin 4.2 g/dL and prothrombin time of 10.6 seconds), hypersplenism (white blood cell count 5000/mm³ and platelet count 102,000/mm³), and cholestasis (total serum bilirubin 1.2 mg/dL, conjugated bilirubin 0.8 mg/dL and total serum bile acids >400 µM). Analysis of urine bile

Keywords: Bile, cholestasis, pediatric, phospholipid

Received: 31.08.2011; **Accepted:** 02.09.2011

Address for correspondence: Benjamin L Shneider, Children's Hospital of Pittsburgh of UPMC, Division of Pediatric Gastroenterology, Hepatology and Nutrition, 4401 Penn Avenue, Pittsburgh, PA – 15224, USA

E-mail: Benjamin.Shneider@chp.edu

Abbreviations: SIFT: sorting intolerant from tolerant

doi: 10.1016/S0973-6883(11)60131-X

Figure 1 Growth parameters. Weight is plotted relative to normative data in the top panel, while height is plotted in the lower panel. Midparental parameters were achieved after institution of ursodeoxycholic acid therapy.

acids did not reveal evidence of any inborn error in bile acid biosynthesis. Review of her liver biopsy was notable for features of portal expansion with fibrous tissue, ductular proliferation, moderate inflammatory infiltrate with lymphocytes and plasma cells, with some evidence of interface hepatitis. Bridging fibrosis and nodule formation was noted. There was no evidence of concentric fibrosis around the bile ducts. Serum IgG was 983 mg/dL and autoantibodies against smooth muscle, nuclear proteins, liver kidney microsome and soluble liver antigen were negative. Magnetic resonance cholangiography did not reveal features of sclerosing cholangitis. A diagnosis of *ABCB4* disease was suspected and ursodeoxycholic acid therapy at 20 mg/Kg/day was instituted.

Biochemical markers of liver disease improved dramatically in the first few months of ursodeoxycholic acid therapy (Figure 2). Growth parameters improved in the first 6 months of therapy (Figure 1). Splenomegaly was less notable, although platelet count steadily diminished. Hepatic synthetic function remained intact. Thirty-six months into her therapy, she is doing well without any liver-related complaints. She continues to show no signs of hepatopulmonary syndrome. Her growth parameters are at the 50th percentile. Surveillance blood work is

Figure 2 Liver biochemistries. Marked improvement in ALT, GGT and total bilirubin (TB) was observed promptly after the institution of ursodeoxycholic acid therapy (2 months after presentation). In contrast, steady worsening in the platelet counts was noted.

notable for minimal, if any, biochemical evidence of liver disease (ALT 28 IU/L, AST 50 IU/L, alkaline phosphatase 280 IU/L, GGT 21 IU/L), intact hepatic synthetic function (albumin 4.5 g/dL and INR 1.2), and hypersplenism (white blood cell count 2800 and platelet count 59,000). Fat soluble vitamin levels in blood have been in the recommended ranges, without any specific supplementation with the exception of vitamin K.

Her family requested specific genetic testing for *ABCB4* disease for the purposes of family planning. Heterozygous missense mutations in the *ABCB4* gene were identified. An A546D mutation was identified, which has been reported in intrahepatic cholestasis of pregnancy.⁴ A second novel mutation, R176W, was detected. This residue is conserved from frog to human and is predicted to be deleterious by polymorphism phenotyping (PolyPhen) and sorting intolerant from tolerant (SIFT). Subsequent genotyping of her parents confirmed that these mutations exist on separate alleles.

DISCUSSION

The diagnosis of Wilson disease can be problematic in designating with certainty. No single diagnostic study establishes this diagnosis and working groups have developed scoring systems to assist clinicians.³ Many of the features of the scoring system are manifestations of copper overload, including most notably urinary and hepatic copper levels. These two prominent features of the scoring system are redundant and diseases associated with secondary copper overload have the potential to lead to misdiagnosis. The scoring systems warn that caution should be exercised in application to individuals with cholestasis, since copper is excreted into bile and cholestasis is associated with copper overload. This issue was not necessarily appreciated early in the evaluation of this patient. Cholestasis was not immediately evident with standard

biochemical testing, since the total bilirubin level was 1.2 mg/dL. Cholestasis was suggested by the elevation in conjugated bilirubin, and only became fully apparent with measurement of serum bile acids. The clinical presentation of this child's disease was atypical for Wilson disease, as cirrhosis at 2 years of age would not have been expected.

The liver biopsy findings in conjunction with the biochemical markers, including a very high GGT level, suggested a biliary type of disease. Sclerosing cholangitis would have been unlikely to have caused this degree of illness at 2 years of age. An MR cholangiogram effectively excluded typical sclerosing cholangitis. Small duct sclerosing cholangitis or *ABCB4* disease were the most likely diagnoses and at the time, ursodeoxycholic acid therapy was appropriate for both. Subsequent investigations have called into question the routine use of ursodeoxycholic acid therapy in adults.⁵ The prompt biochemical and clinical response to ursodeoxycholic acid therapy supported the veracity of these diagnoses.^{6,7} At the time, routine genotyping for *ABCB4* disease was not available.

ABCB4 disease is the result of diminished canalicular excretion of phospholipids.⁸ In this disease biliary bile acids are not micellized by phospholipids and cholesterol, resulting in extracellular injury to the bile canaliculus and to cholangiocytes. Variable disease results from *ABCB4* disease, with fairly good correlation between genotype, predicted functional effects and clinical phenotype.⁹ Functionally severe mutations are associated with progressive biliary cirrhosis that often leads to end-stage liver disease in the first decade of life. It is curious that a Wilsonian form of *ABCB4* disease has been reported.¹⁰ Functionally, more mild mutations yield much more protean clinical manifestations, including nonspecific chronic hepatitis, intrahepatic cholestasis of pregnancy, intrahepatic lithiasis and a more slowly progressive biliary cirrhosis.⁹ In many of these circumstances, a pronounced biochemical response has been noted in response to ursodeoxycholic acid therapy. The presumption has been that ursodeoxycholic acid leads to a change in bile composition to one that is more hydrophilic and less toxic to cholangiocytes. It appears that this biochemical response is associated with a slowing of the progression of the liver disease. Absolute proof of this observation will be difficult to obtain as it is unlikely that randomized trials will be performed. In this case, liver biochemistries were markedly improved and were associated with a remarkable improvement in growth parameters. Despite these reassurances, thrombocytopenia has worsened, independent of changes in spleen size. We remain cautiously optimistic about her prognosis, although suspect that she will ultimately require liver transplantation.

Genotyping for specific genetic liver diseases is changing diagnostic approaches in pediatric hepatology. Clinicians need to be cognizant of the advantages, disadvantages and limitations of genetic analyzes. The absence of relevant

changes in the gene of interest suggests but does not prove that the diagnosis in question has been excluded. One must consider the potential limitations of genotyping, including sequencing errors and the inability to identify some intronic and promoter-based mutations. In addition, large genetic deletions may be difficult to identify if they affect a single allele. A common problem that is encountered is the identification of presumed irrelevant polymorphisms and/or the finding of unique mutations of unclear biologic significance. In our case, a unique mutation was identified—the conservation of the residue amongst many species and computer predictions of its functional significance supported the premise of its biologic significance. These represent logical conclusions but are not easily supported by in vitro functional correlates or confirmation in additional individuals with similar disease manifestations.

In summary, we have described a young child who presented with cirrhosis and copper overload secondary to cholestasis related to a novel *ABCB4* genotype. She has shown a biochemical and clinical response to ursodeoxycholic acid therapy. Clinicians need to consider the effect of cholestasis on the diagnostic criteria for Wilson disease, in order to arrive at the correct diagnosis and therapeutic approach.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Frydman M. Genetic aspects of Wilson's disease. *J Gastroenterol Hepatol* 1990;5:483–90.
2. Nicastro E, Ranucci G, Vajro P, Vegnente A, Iorio R. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. *Hepatology* 2010;52:1948–56.
3. Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003;23:139–42.
4. Dixon P, Weerasekera N, Linton K, et al. Heterozygous MDR3 missense mutation associated with intrahepatic cholestasis of pregnancy: evidence for a defect in protein trafficking. *Hum Mol Genet* 2000;9:1209–17.
5. Lindor KD, Kowdley KV, Luketic VA, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009;50:808–14.
6. Miloh T, Arnon R, Shneider B, Suchy F, Kerker N. A retrospective single-center review of primary sclerosing cholangitis in children. *Clin Gastroenterol Hepatol* 2009;7:239–45.
7. Stapelbroek JM, van Erpecum KJ, Klomp LW, Houwen RH. Liver disease associated with canalicular transport defects: current and future therapies. *J Hepatol* 2010;52:258–71.
8. Smit JJM, Schinkel AH, Oude Elferink RPJ, et al. Homozygous disruption of the murine *mdr2* P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993;75:451–62.
9. Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to *ABCB4* gene mutations: pathophysiology and clinical aspects. *Semin Liver Dis* 2010;30:134–46.
10. Anheim M, Chamouard P, Rudolf G, et al. Unexpected combination of inherited chorea-acanthocytosis with MDR3 (*ABCB4*) defect mimicking Wilson's disease. *Clin Genet* 2010;78:294–5.