Novel mutations in NOTCH2 gene in infants with neonatal cholestasis

Eliana Shaul,1 Debora Kogan-Liberman,2 Stephanie Schuckalo,3 Dominique Jan,4 Michelle Ewart,5 Trang Nguyen,2 Mercedes Martinez,6 Nadia Ovchinsky,2,3
1Department of Pediatrics, Children’s Hospital at Montefiore, Bronx, NY; 2Division of Pediatric Gastroenterology, Hepatology and Nutrition, Children’s Hospital at Montefiore, Bronx, NY; 3Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Goryeb Children’s Hospital - Atlantic Health System, Morristown, NJ; 4Department of Pediatric Surgery, Children’s Hospital at Montefiore, Bronx, NY; 5Division of Surgical Pathology, Montefiore Medical Center, Bronx, NY; 6Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Morgan Stanley Children’s Hospital of New York, NY, USA

Abstract

One cause of neonatal cholestasis (NC) is paucity of intrahepatic bile ducts which can be associated with Alagille syndrome or non-syndromic. Alagille syndrome is caused by autosomal dominant mutations in the Notch signaling pathway ligand Jagged1 in 94% of patients with NC.5 SIFT predicts whether an amino acid substitution is likely to affect protein function based on sequence homology and similarity between the alternate amino acids and then predicts if the amino acid change is either ‘tolerated’ or ‘deleterious’. PolyPhen-2 predicts the effect of an amino acid substitution on the structure and function of a protein using sequence homology, 3D structures where available, and a number of other databases and tools and then provides a qualitative prediction of ‘probably damaging’, ‘possibly damaging’, ‘benign’ or ‘unknown’. This study was deemed exempt by The Albert Einstein College of Medicine Institutional Review Board.

Case Report

Five male infants with NC between the age of 2 and 8 weeks were found to have one copy of VOUS in NOTCH2 gene (Table 1). None of the infants had known risk factors for cholestasis. They all presented with jaundice, acholic stools and without growth failure. Patients 1 and 2 were siblings with identical mutations. Liver biopsy was done in 3 patients demonstrating paucity of bile ducts in 2 patients (Figure 1) and mild ductular proliferation in the third. In all patients SIFT predicted the mutations to be deleterious and PolyPhen predicted them to be probably damaging. Further workup for other features of ALGS, including an echocardiogram, eye exam and X-Ray of the spine, were all within normal limits. Additionally, workup for common etiologies of NC including infectious, anatomic, metabolic, and genetic etiologies was unremarkable. All patients demonstrated...
improvement in liver disease over time and remain asymptomatic.

Discussion

NC is caused by diminished flow and excretion of bile. Symptoms typically include jaundice, dark urine, acholic stools and hepatomegaly. Cholestasis can occur due to infectious, genetic, anatomic and metabolic causes, generally caused by impairment of hepatobiliary transport, intermediary metabolism, storage disorders or bile duct dysgenesis. PIBD is one cause of NC and is defined by a specimen from a liver biopsy shows a loss of intrahepatic bile ducts in more than 50% of portal tracts in a specimen that contains at least 10 portal tracts. PIBD can be part of a genetic syndrome, ALGS or can caused other rare metabolic diseases, infections such as CMV, or can be idiopathic non-syndromic paucity. A biopsy in patients with ALGS typically demonstrates paucity of the intrahepatic bile ducts, however in newborns with ALGS, bile duct paucity is not always present and instead ductal proliferation can be found. The diagnosis of ALGS requires the presence of bile duct paucity with three of five major clinical features including liver disease, vertebral abnormalities, congenital heart defects, ocular anomalies and characteristic facial features. ALGS is an autosomal dominant inherited disorder with highly variable expressivity, therefore the disease penetration and severity of the affected organs can vary significantly.

Notch pathway interactions are critical for determination of cell fates and differentiation in early development. The Notch system includes four transmembrane Notch receptors (Notch 1, 2, 3, 4) and two types of ligands Jagged (Jag 1, 2) or Delta-like (Dil 1, 3, 4). The Notch pathway is involved in several stages of bile duct morphogenesis including in the expression of cholangiocytes-specific markers committing cells to the biliary lineage.

In 94% with ALGS, mutations in JAG1 are found, while mutations in NOTCH2 have been described in a small number of patients who met diagnostic criteria for ALGS without JAG1 mutations. In one study, Kamath et al describe a cohort of JAG1-negative individuals with clinical features suggestive of ALGS screened for NOTCH2 mutations. Eight patients with a NOTCH2 mutations were identified and only three met classic criteria for ALGS. Of the remaining five individuals, four had two typical ALGS diagnostic features and one
patient had bile duct paucity with no other syndromic features. This study found that in their patients the variety of clinical features associated with NOTCH2 mutations differed from JAG1 mutations, with a lower prevalence of butterfly vertebrae and facial features.

Conclusions

These five patients, along with one patient described by Kamath et al., suggest that NOTCH2 mutations may be related to isolated NC or PIBD without other features of ALGS. Since the Notch pathway is involved in bile duct morphogenesis, these cases stipulate that NOTCH2 mutations may result in hypoplastic biliary system and intrahepatic bile duct paucity. Furthermore, this series suggests that NOTCH2 mutations may provide the genetic basis to explain the clinical finding in infants with isolated neonatal cholestasis without other features of ALGS. Screening for NOTCH2 mutations in patients with NC is warranted and in those with PIBD even if they only fulfil partial criteria for ALGS. Validation of these findings in a larger human cohort and further characterization of the NOTCH2 variants in an animal model is especially important to understand the clinical application of these results. Further investigation will need to be done in order to determine whether these NOTCH2 variants are truly deleterious as the current data presented are insufficient to determine causality of these variants in neonatal cholestasis. Increasing availability of genetic testing and ability to link the clinical finding with previously unrecognized mutations provide a platform for more precise and less invasive approach to achieve a diagnosis in this vulnerable population.

References

1. Feldman AG, Sokol RJ. Neonatal cholestasis. Neoreviews 2013;14.
2. Zahmatkeshan M, Geramizadeh B, Haghighat M, Enteshari, H. Paucity of Intrahepatic Bile Ducts in Neonates: the First Case Series from Iran. Iran J Pediatrics 2013;23:65-70.
3. Kenny AP, Crimmins NA, Mackay DJ, et al. Concurrent course of transient neonatal diabetes with cholestasis and paucity of interlobular bile ducts: A case report. Pediatr Dev Pathol 2009;12:417-20.
4. McDaniel R, Warthen DM, Sanchez-Lara PA, et al. NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway. Am J Hum Genet 2006;79:169-73.
5. Flanagan SE, Patch AM, Ellard S. Using SIFT and PolyPhen to predict loss-of-function and gain-of-function mutations. Genet Test Mol Biomarkers 2010;14:533-77.
6. Suchy FJ. Neonatal cholestasis. Pediatr Rev 2004;25:388-96.
7. Suchy FJ. Clinical problems with developmental anomalies of the biliary tract. Semin Gastrointest Dis 2003;14:156-64.
8. Saleh M, Kamath BM, Chitayat D. Alagille syndrome: clinical perspectives. Appl Clin Genet 2016;9:75-82.
9. Piccoli DA, Spinner NB. Alagille syndrome and the Jagged1 gene. Semin Liver Dis 2001;21:525-34.
10. Kamath BM, Bason L, Piccoli DA, et al. Consequences of JAG1 mutations. J Med Genet 2003;40:891-5.
11. Morell CM, Strazzabosco M. Notch signaling and new therapeutic options in liver disease. J Hepatol 2014;60:885-90.
12. Guegan K, Stals K, Day M, et al. JAG1 mutations are found in approximately one third of patients presenting with only one or two clinical features of Alagille syndrome. Clin Genet 2012;82:33-40.
13. Kamath BM, Bauer RC, Loomes KM, et al. NOTCH2 mutations in Alagille syndrome. J Med Genet 2012;49:138-