Novel mutations in *NOTCH2* gene in infants with neonatal cholestasis

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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 and mutations in the *NOTCH2* receptor in <1% of patients. This is a retrospective case series studying infants with neonatal cholestasis found to have variants of unknown significance (VOUS) in *NOTCH2*. Sorting intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen) were utilized to predict a damaging effect. Five infants with NC without other features of Alagille syndrome were found to have one copy of a VOUS in *NOTCH2*, predicted to be damaging by SIFT and PolyPhen. Our cases support the notion that *NOTCH2* mutations may result in hypoplastic biliary system. Further characterization of these variants is important to assist with our clinical approach to NC.

Introduction

Impaired flow and excretion of bile in the newborn period results in accumulation of biliary components and leads to neonatal cholestasis (NC).[1] Paucity of intrahepatic bile ducts (PIBD) is one cause of NC and can be part of a an autosomal dominant familial cholestatic syndrome called Alagille Syndrome (ALGS)[2] or can be non-syndromic.[3] In 94% of patients with ALGS, mutations in the gene for the Notch signaling pathway ligand Jagged1 (*JAG1*) are found. Furthermore, mutations in *NOTCH2*, a receptor in the Notch signaling have been found in patients with ALGS.[4] We report 5 patients, including 2 siblings (patients 1&2), with NC without any other features of ALGS, who were found to have novel variants of unknown significance (VOUS) in the *NOTCH2* gene.

Methods

This is a retrospective case series of 5 patients with NC. Data was obtained through a chart review, including electronic medical records, imaging and pathology specimens. Genetic testing for known pediatric cholestatic disorders was done via Emory Genetics Laboratory (EGL) Genetic Cholestasis Panel. Direct sequencing of the amplified captured regions was performed using next generation short base pair read sequencing (NGS). Exons with inadequate quality or coverage by NGS were assessed with Sanger sequencing. We utilized sorting intolerant from tolerant (SIFT) and polymorphism phenotyping (PolyPhen), which are silico bioinformatic tools that predict the likelihood of pathogenicity of missense variants.[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 *NOTCH 2* 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

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improvement in liver disease over time and remain asymptomatic.

Discussion

NC is caused by diminished flow and excretion of bile.[1] 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.[6] 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.[7] 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.[2,3] A liver 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.[8] 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.[9] ALGS is an autosomal dominant inherited disorder with highly variable expressivity, therefore the disease penetration and severity of the affected organs can vary significantly.[10]

Notch pathway interactions are critical for determination of cell fates and differentiation in early development**.** The Notch system includes of four transmembrane Notch receptors (Notch 1, 2, 3, 4) and two types of ligands Jagged (Jag 1, 2) or Delta-like (Dll 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.[11]

In 94% with ALGS, mutations in *JAG1* are found,[4] while mutations in *NOTCH2* have been described in in a small number of patients who met diagnostic criteria for ALGS without *JAG1* mutations.[9,12] 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

Table 1. Patient characteristics.

Patient	Age (weeks)	Presenting symptoms	Total/direct bilirubin, mg/dL	Gamma-glutamyl transferase, U/L	Abdominal ultrasound	HIDA Scan	Cholangiogram	Liver Biopsy	NOTCH2 Mutation
1	3	Jaundice, acholic stools	6.1/3.4	222	Normal liver size and echotexture. No dilated intrahepatic or extrahepatic bile ducts. Gallbladder present.	No gall bladder or bowel uptake after 24 hours	Intra-operative cholangiogram: Patent intrahepatic and extrahepatic biliary system	Paucity of bile ducts and mild ductular proliferation	NOTCH2 VOUS c.5314G>A (p.E1772K)
2	8	Jaundice, acholic stools	6.7/4.5	638	Mild hepatomegaly with contracted but present gallbladder	No gall bladder or bowel uptake after 24 hours	Percutaneous cholangiogram: Patent intrahepatic and extrahepatic biliary system	Paucity of bile ducts	NOTCH2 VOUS c.5314G>A (p.E1772K)
3	2	Jaundice, acholic stools, feeding difficulty	10/2.5	296	Normal liver size and echotexture. No dilated intrahepatic or extrahepatic bile ducts. Gallbladder present.	Prompt and homogeneous radiotracer uptake in the liver with prompt excretion into the bowel	Not done	Not done	NOTCH2 VOUS c.1847G>T (p.C616F)
4	4	Jaundice, acholic stools, hepatomegaly	4.6/0.9	224	Normal liver size and echotexture. No dilated intrahepatic or extrahepatic bile ducts. Gallbladder present.	Not done	Not done	Not done	NOTCH2 VOUS c.2102T>A (p.C701E)
5	2	Jaundice, acholic stools	5.7/4.8	217	Normal liver in size and echogenicity. Gallbladder was not visualized.	Prompt and homogeneous radiotracer uptake in the liverwith prompt excretion into the bowel	Not done	Mild ductular proliferation	NOTCH2 VOUS c.4699C>T (p.R1567W)

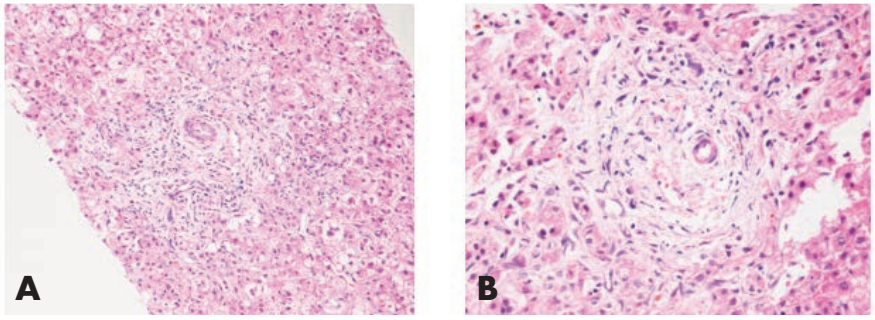

Figure 1. Histological findings demonstrating PIBD in patient 1. A) Portal area with mid chronic inflammation with visible hepatic artery branch and portal vein branches without any native bile ducts. There are surrounding giant cell transformation of hepatocytes. B) Another portal area showing hepatic artery and portal vein without a native bile duct.

patient had bile duct paucity with no other syndromic features.[13] 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 basic to explain the clinical finding in infants with isolated neonatal cholestasis without other features of ALGS. It is also crucial to consider that the highly variable expression and reduced penetrance seen with ALGS could be influencing clinical presentation seen in our patients. 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 finding 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.

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