Research Article

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Mutations in *DCDC2* (doublecortin domain containing protein 2) in neonatal sclerosing cholangitis

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Background & Aims: Neonatal sclerosing cholangitis (NSC) is a severe neonatal-onset cholangiopathy commonly leading to liver transplantation (LT) for end-stage liver disease in childhood. Liver biopsy findings histopathologically resemble those in biliary atresia (BA); however, in NSC extrahepatic bile ducts are patent, whilst in BA their lumina are obliterated. NSC is commonly seen in consanguineous kindreds, suggesting autosomal recessive inheritance.
Methods: From 29 NSC patients (24 families) identified, DNA was available in 24 (21 families). Thirteen (7 male) patients (12 families) of consanguineous parentage were selected for whole exome sequencing. Sequence variants were filtered for homozygosity, pathogenicity, minor allele frequency, quality score, and encoded protein expression pattern.
Results: Four of 13 patients were homozygous and two were compound heterozygous for mutations in the doublecortin domain containing 2 gene (*DCDC2*), which encodes DCDC2 protein and is expressed in cholangiocyte cilia. Another 11 patients were sequenced: one (with one sibling pair) was compound heterozygous for *DCDC2* mutations. All mutations were protein-truncating. In available liver tissue from patients with *DCDC2* mutations, immunostaining for human DCDC2 and the ciliary protein acetylated alpha-tubulin (ACALT) showed no expression (n = 6) and transmission electron microscopy found that cholangiocytes lacked primary cilia (n = 5). DCDC2 and ACALT were expressed in NSC patients without *DCDC2* mutations (n = 22). Of the patients carrying *DCDC2* mutations, one died awaiting LT; five came to LT, of whom one died 2 years later. The other 4 are well.
Conclusion: Among 24 NSC patients with available DNA, 7 had mutations in *DCDC2* (6 of 19 families). NSC patients in substantial proportion harbour mutations in *DCDC2*. Their disease represents a novel liver-based ciliopathy.
Lay summary: Neonatal sclerosing cholangitis (NSC) is a rare genetic form of liver disease presenting in infancy. Through next generation sequencing we identified mutations in the gene encoding for doublecortin domain containing 2 (DCDC2) protein in a group of NSC children. DCDC2 is a signalling and structural protein found in primary cilia of cholangiocytes. Cholangiocytes are the cells forming the biliary system which is the draining system of the liver.

Keywords: Neonate; Cholangiopathy; Doublecortin domain containing protein 2; Ciliopathy; Acetylated alpha-tubulin.
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Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; ACALT, acetylated alpha-tubulin; BA, biliary atresia; Ca^{2+}, calcium; CHF, congenital hepatic fibrosis; DCDC2, doublecortin domain containing protein 2; DCX, doublecortin gene family; ERCP, endoscopic retrograde cholangiopancreatography; ERK, extracellular signal-regulated kinase; GGT, γ-glutamyltransferase; IFT, intraflagellary transport system; KCH, King's College Hospital; LT, liver transplantation; MAP, microtubule-associated protein; MDR3, multidrug resistance protein 3; MRCP, magnetic resonance cholangiopancreatography; NPHP-RC, nephronophthisis-related ciliopathies; NSC, neonatal sclerosing cholangitis; PSC, primary sclerosing cholangitis; TEM, transmission electron microscopy; WES, whole exome sequencing.

Introduction

Neonatal sclerosing cholangitis (NSC) is a rare form of severe liver disease first reported in 8 children presenting in early infancy with jaundice, hepatosplenomegaly, pale stools, and high serum γ-glutamyltransferase (GGT) activity [1]. Ductular proliferation, moderate portal tract inflammation, and fibrosis were found at liver biopsy. Percutaneous cholangiography confirmed intrahepatic cholangiopathy in all; 2 had earlier undergone laparotomy to exclude biliary atresia (BA). Most developed biliary cirrhosis.

Three patients were born to consanguine parents, suggesting recessive inheritance. The term NSC was first used for high GGT neonatal-onset cholangiopathy in another consanguine sibling pair. Biliary cirrhosis required liver transplantation (LT) for survival [2]. A distinct hepatorenal disorder was later suggested in 2 siblings with renal disease, high GGT activity, and, on endoscopic retrograde cholangiopancreatography (ERCP) and liver biopsy, early onset changes like those of primary sclerosing cholangitis [3]. Cholangiopathy in children has been attributed to immune dysregulation (autoimmune sclerosing cholangitis, immunodeficiency or Langerhans cell histiocytosis [4]) and to single-gene disorders (deficiency of multidrug associated protein 3 (MDR3), encoded by *ABCB4* [5], claudin-1 deficiency [6] or Kabuki syndrome [7,8]); as with BA, it also may have multiple different causes [9].

The aim of this study was to identify genes mutated in NSC patients seen at King's College Hospital (KCH). We describe the clinical and laboratory features, presentation, and disease progression of NSC in these patients; the process and results of whole exome sequencing (WES) in a subgroup of these patients, with Sanger sequencing confirmation of candidate gene mutations and selective sequencing of candidate genes, when possible, in the remaining patients; and the findings within liver and biliary tract on immunohistochemical assessment of encoded protein and comparison-protein expression as well as on ultrastructural study.

Patients and methods

Patients

The diagnosis of NSC was assigned to 29 patients (24 families) whose disorder clinically presented during infancy; who had cholestasis with elevated GGT; and in whom cholangiopathy was demonstrated on histopathologic study or imaging. Exclusion criteria were evidence of ichthyosis-like skin lesions, extrahepatic abnormalities suggesting Alagille syndrome, mutations in *ABCB4*, or immune dysregulation. Patients had normal serum immunoglobulin values (immunoglobulin (Ig)M, IgG, IgA), lacked demonstrable autoantibodies (antinuclear, -smooth muscle, -liver – kidney microsome, -mitochondrial, -gastric parietal cell), and had normal complement levels (C3/C4).

Histologic features of cholangiopathy and cholestasis, present in all available specimens (28 patients), included porto-septal bridging fibrosis, ductular reaction, hepatocellular metallothionein deposits, and intralobular bile pigment accumulations (Fig. 1A). MDR3 expression was demonstrated immunohistochemically (Fig. 1F, inset) in all specimens. Radiological features included irregular dilatation and strictures in intrahepatic or extrahepatic bile ducts, consistent with a cholangiopathy.

Stored blood or DNA was available in 24 patients (19 families). Blood was retrieved for WES from the Paediatric Liver Centre biobank for 13 children (12 families) chosen for parental consanguinity and availability of DNA suitable for next generation sequencing (Supplementary Table 1). The remaining 11 patients subsequently underwent Sanger sequencing of *DCDC2*. Parental or patient consent had previously been obtained for research investigation in accordance with institutional guidelines. Ethical-review committee approval for this specific study was obtained, with samples anonymised before use.

Whole exome sequencing

WES was undertaken using the Roche Nimblegen SeqCap EZ Human Exome Library v2.0, as per manufacturer's protocol. Initial analysis focused on finding variants distributed in a pattern consistent with autosomal recessive disease inheritance. WES to permit cataloguing of genetic variation in patients followed published protocols [10]. Variants were annotated with Variant Effect Predictor and loaded into Gemini software [11]. Variants with minor allele frequency >1% in the 1000 Genome or the Exome Sequencing Project data were excluded, as were intergenic variants and variants that were flagged as low quality or potential false-positives (quality scores ⩽30; long homopolymer runs >5; low quality by depth <5; occurrence within a cluster of single-nucleotide polymorphisms). Variants of interest (see above) were prioritised for biological relevance.

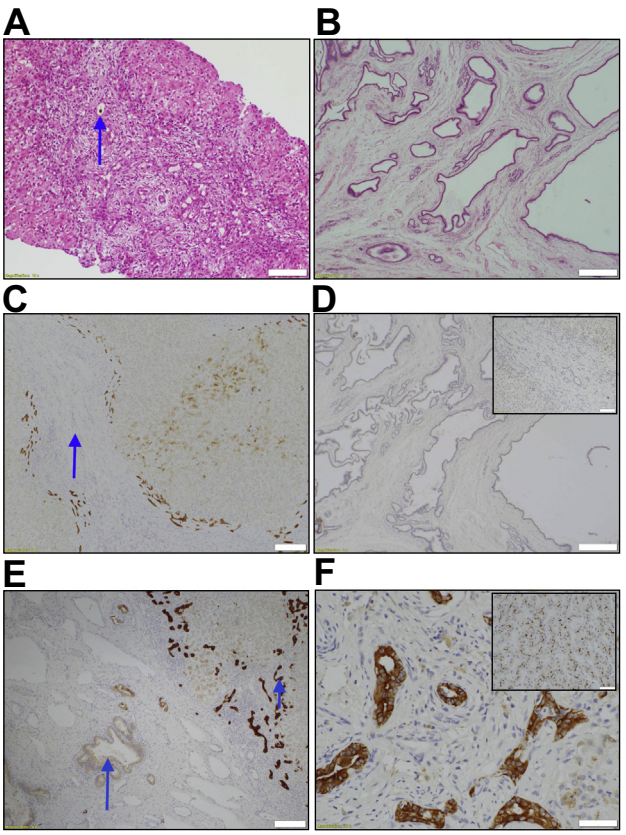

Fig. 1. Liver biopsy histology and immunohistochemistry in NSC patients with and without mutations in *DCDC2*. (A) Liver biopsy at 4 months from an NSC patient with *DCDC2* mutations (patient 6) showing expansion of portal areas with ductal bile plugs (arrow) and ductular reaction (H&E, magnification 100×. Calibration bar = 100 μm). (B) Variable ectasia of perihilar bile ducts in a hepatectomy specimen from an NSC patient with *DCDC2* mutations (patient 4, H&E, magnification 20×. Calibration bar = 500 μm). (C) In the periphery of a hepatectomy specimen from an NSC patient with *DCDC2* mutations, cytokeratin 7 (CK7) immunostaining demonstrates a ductular reaction, but no bile ducts are detected in portal areas broadened by fibrosis (arrow). Aberrant expression of CK7 is seen within the lobule, indicating chronic cholestasis (patient 4, magnification 40×. Calibration bar = 200 μm). (D) DCDC2 immunostaining in NSC patients with *DCDC2* mutations demonstrated lack of expression in large perihilar bile ducts as well as small interlobular bile ducts (main image from patient 4, absence of DCDC2 immunostaining in perihilar bile ducts, magnification 20×. Calibration bar = 500 μm. Inset from patient 5, liver biopsy at 9 weeks, absence of DCDC2 immunostaining in interlobular bile ducts, magnification 100×. Calibration bar = 100 μm). (E) A hepatectomy specimen from an NSC patient with no *DCDC2* mutations shows weak and focal DCDC2 staining in large perihilar bile ducts (long arrow) and strong diffuse staining in neoductules (short arrow) (patient 12, magnification 40×. Calibration bar = 200 μm). (F) Strong cytoplasmic and apical biliary epithelial expression of DCDC2 is seen within interlobular bile ducts from an NSC patient without *DCDC2* mutations (main image, patient 17, liver biopsy at 35 weeks, DCDC2 immunostaining, magnification 200×. Calibration bar = 50 μm). Multidrug resistance protein 3 immunostaining demonstrated canalicular expression in NSC patients with *DCDC2* mutations (inset, patient 3, liver biopsy at 8 weeks, magnification 200×. Calibration bar = 50 μm).

Sanger sequencing

Sanger sequencing confirmed variants identified by WES in the first set of patients. Forward and reverse primers were designed and annealing temperatures were set for genes of interest (Supplementary Table 2). PCR amplification and sequencing reactions were performed using standard protocols [12,13].