Liver Disease and Risk of Hepatocellular Carcinoma in Children With Mutations in *TALDO1*

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Mutations in the transaldolase 1 (*TALDO1*) gene have been described in a limited number of cases. Several organs can be affected and clinical manifestations are variable, but often include liver dysfunction and/or hepatosplenomegaly. We report 4 patients presenting with liver disease: 2 with early-onset hepatocellular carcinoma (HCC). Patients with cholestasis and mutations in *TALDO1* were identified by next-generation sequencing. Clinical, laboratory, and histological data were collected. Four (1 male) patients were identified with variants predicted to be damaging in *TALDO1*. Three patients were homozygous (two protein truncating/one missense mutations), 1 one was compound heterozygous (two missense mutations). Median age at presentation was 4 months (range, 2-210 days) with jaundice (3), hepatosplenomegaly (3), and pancytopaenia (1). The diagnosis was corroborated by detection of minimal transaldolase enzyme activity in skin fibroblasts in two cases and raised urine polyols in the third. Three patients underwent liver transplantation (LT), 2 of whom had confirmed HCC on explanted liver. One patient suddenly died shortly after LT. The nontransplanted case has a chronic liver disease with multiple dysplastic liver nodules, but normal liver biochemistry and alpha-fetoprotein. Median follow-up was 4 years (range, 1-21). *Conclusion:* Transaldolase deficiency can include early-onset normal gamma-glutamyltransferase liver disease with multisystem involvement and variable progression. Patients with this disease are at risk of early-onset HCC and may require early LT. (*Hepatology Communications* 2022;6:473-479).

Transaldolase deficiency (OMIM 606003) is a rare inborn error of metabolism caused by defects in the *TALDO1* gene, encoding one enzyme of the pentose phosphate pathway.[1] Within this pathway, transaldolase is important for the generation of erythrose 4-phosphate, which is necessary for synthesis of aromatic amino acids. Transaldolase deficiency has been described in more than 34 cases (25 families), and 11 different mutations in *TALDO1* have been reported.[2-4] Clinical manifestations vary but can include liver dysfunction and/or hepatosplenomegaly, growth retardation, congenital heart disease, and endocrine dysfunction.[5-9]

We report here 4 patients, genetically diagnosed with transaldolase deficiency, manifesting liver disease, and in two cases early-onset liver carcinogenesis.

Patients and Methods

Data including demographics, clinical information, laboratory, radiological (Table 1), and histological/immunohistochemical (Fig. 1) findings as well as longitudinal follow-up (Table 1) were collected from electronic patient records. Where available, parental DNA samples were also tested, confirming heterozygosity.

The data were collected as approved by the King's College Hospital Pediatric Liver Biobank (Reg. No.: CH0011/19/DH) and research ethics approval (REC reference 18/WA/0009, IRAS project ID 238002).

GENETIC TESTING

Patient 1

Genetic testing for cholestatic liver disease was undertaken using targeted next-generation sequencing (NGS), which at the time consisted of mutations in *ABCB11* (adenosine triphosphate binding cassette subfamily B member 11), *ABCB4*, *ATP8B1* (ATPase phospholipid transporting 8B1), *TJP2* (tight junction protein 2), *BAAT* (bile acid–CoA:amino acid N-acyltransferase), *NOTCH2* (notch receptor 2), and *JAG1* (jagged canonical notch ligand 1). As diagnosis was not made, whole-exome sequencing (WES) was performed. Sequencing and bioinformatic analysis were performed at the Center for Mendelian Genomics at the University of Washington (Seattle, WA). The sample library was prepared using Roche Nimblegen SeqCap EZ Human Exome Library v2.0 (Basel, Switzerland) and sequenced on the Illumina HiSeq 2500 sequencer (San Diego, CA).

Using the Gemini software package,[10] variant effect predictor–annotated variants under an autosomal recessive model were filtered for depth greater than 6, genotype quality greater than 20, and not seen in Exome Sequencing Project 6500 (V2), 1000 Genomes (Phase 3), and Exome Aggregation Consortium (ExAC) (v.03) with minor allele frequency greater than 0.005.

Patients 2, 3, and 4

Patients 2 and 3 underwent clinical genetic testing for cholestasis using a panel of 28 genes, including *TALDO1*.[11] Patient 4 underwent genetic investigations following liver transplantation (LT) as described.[12]

Results

PATIENT 1

A first-born male infant of consanguineous parents of Turkish origin presented with conjugated jaundice on the second day of life. Blood tests also revealed pancytopenia, requiring red blood cell transfusion, while bone marrow aspirate showed hypercellularity with increased erythropoiesis. No dysmorphic features were identified, and there was no family history of liver disease. At 5 months of age, he was admitted to hospital after a minor injury. Abdominal computed tomography (CT) scan showed hepatosplenomegaly, multiple gallstones, and a subcutaneous abscess in the xiphoid process area. Contrast echocardiography

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TABLE 1. LABORATORY DATA ON ALL 4 PATIENTS AT TIME OF PRESENTATION AND AT LAST PRE-LT FOLLOW-UP (WHERE AVAILABLE)

Laboratory Results (Normal Values)	Pt 1	Pt 2	Pt 3	Pt 4
AST (10-77 IU/L)	217/138	47/34	105	142/197
ALT (5-55 IU/L)	85/74	54/21	n/a	76/130
Total bilirubin (3-20 μmol/L)	50/27	3/3	106	73/23
GGT (1-55 IU/L)	28/51	33/26	34	13/16
ALP (129-291 IU/L)	1042/1041	264/227	1,609	832/879
Albumin (35-50 g/L)	38/37	42/48	30	34/34
AFP (<6 kIU/L)	1,190/172	32/6	1,170	2,328/185
Hemoglobin (115-165 g/L)	100/141	121/119	74	105/111
Platelet count (150-550 × 10^9/L)	78/165	205/140	91	68/94
White cell count (5-19 × 10^9/L)	5.2/26.1	8.66/8	3.51	5.39/8.45
INR (0.9-1.2)	2.2/1.62	0.97/0.98	2.35	1.89/1.49
Calcium (2.1-2.6 mmol/L)	2.1/2.33	2.49/	1.84	2.43/2.25
Urea (2.5-6.5 mmol/L)	3.1/4.2	5.1/5.7	2.6	3.2/6.2
Creatinine (45-120 μmol/L)	34/9	26/31	42	8/11
Cortisol (130-580 nmol/L)	198	239	1,026	N/A
TSH (0.3-5.5 mIU/L)	4.1	5.2	2.3	1.4
fT4 (9-25 pmol/L)		15.6	21	14.1
Vitamin D (>50 nmol/L)	16.9/23.8	65/54	6.4/35	28/8.1
Mutation zygosity	Homozygous	Compound heterozygous	Homozygous	Homozygous
Nucleotide change(s)	c.412C>T	c.542C>T, c.560C>T	c.695_696del	c.574C>T
Amino acid change(s)	p.(Arg138Ter)	p.(Ala181Val), p.(Ser187Phe)	p.(Ile232SerfsTer19)	p.(Arg192Cys)
Enzyme activity/urinary polyols	N/A	Skin fibroblasts activity; 1 nmol/(mg protein) NR: 14-24	Skin fibroblasts activity; 1 nmol/(mg protein) NR: 14-24	Urine polyols raised[10]

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase, AST, aspartate aminotransferase, fT4, free thyroxine 4; INR, international normalized ratio; N/A, not available; NR, normalized ratio; TSH, thyroid stimulating hormone.

showed an enlarged right ventricle, mild pulmonary stenosis with patent foramen ovale, and patent ductus arteriosus with evidence of intrapulmonary shunting. Staphylococcus aureus was identified in the abscess, which was drained. Nitro-blue tetrazolium test excluded chronic granulomatous disease. On liver biopsy, canalicular cholestasis, macrovesicular steatosis, and fibrosis with nodular transformation were observed (Fig. 1). A few weeks later the child was hospitalized with decompensated liver disease, coagulopathy, cholestasis, and established hepato-pulmonary syndrome (Table 1). Urine bile acid profile did not suggest an inborn error of bile acid synthesis, and serum bile acids (218 μmol/L [nv < 14]) confirmed cholestasis. Liver ultrasound scan (USS) demonstrated dilated bile ducts, focal liver lesions, and splenomegaly. At the age of 5 months, his serum alpha-fetoprotein (AFP) was 1,190 kIU/L but decreased to 172 kIU/L

by the age of 10 months. Additional investigations did not suggest primary immunodeficiency and have excluded cystic fibrosis.

The patient was listed for LT and received a cadaveric graft at the age of 11 months. Histology of the explanted liver demonstrated four dysplastic nodules and two well-differentiated hepatocellular carcinomas (HCCs) on a background of cirrhosis (Fig. 1). On day 1 following LT, the patient developed ventricular fibrillation and suddenly died of cardiac arrest. Autopsy suggested that the death was secondary to acute heart failure. Cardiac conductive tissue did not show any macroscopic or histological abnormality.

Sequencing of seven known cholestasis genes identified a single heterozygous nucleotide change in exon 23 of *ABCB11* gene: c.2854G>A; p.(Lys952Arg). Although this variant is predicted to be potentially
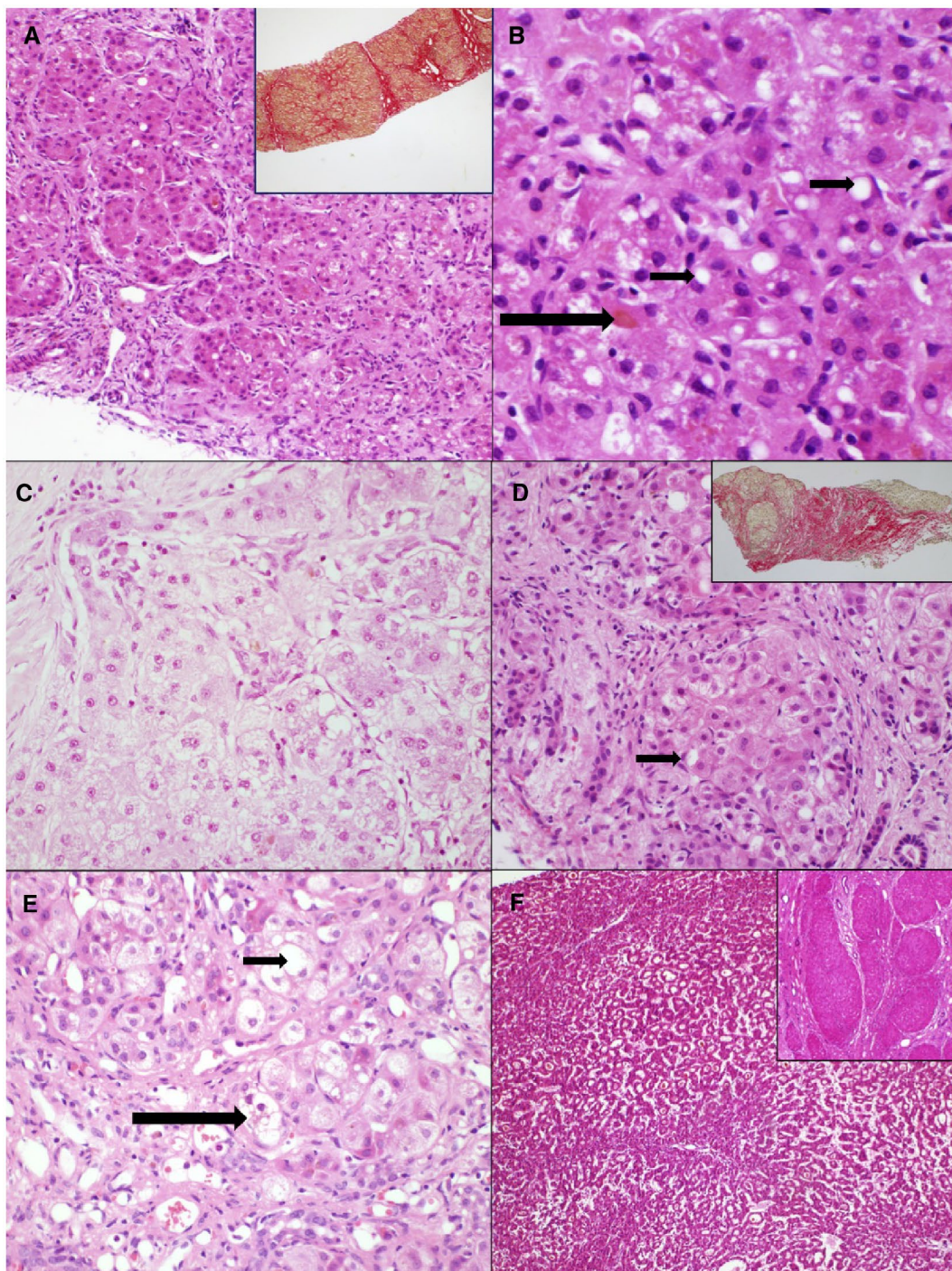
FIG. 1. Patient 1's liver histology at 6 months of age demonstrated extensive perisinusoidal and bridging fibrosis with nodular transformation. (A) Inset: Sirius red stain (×40 magnification). Main image: hematoxylin and eosin (H&E; ×100 magnification). (B) There was mild macrovesicular steatosis (short arrows) and canalicular cholestasis (long arrows) (H&E, ×200 magnification). Patient 1 underwent LT at 11 months of age. (C) The explant demonstrated dysplastic nodules and several well differentiated HCCs (H&E, ×200 magnification) in a background of cirrhosis. (D) Patient 2's liver biopsy at 9 months of age showed cirrhosis (inset: sirius red stain, ×100 magnification) and moderate steatosis (arrow) (main image :H&E, ×200 magnification). (E) Patient 3's hepatectomy at 5 months of age demonstrated cirrhosis and mixed macrovesicular (short arrow) and microvesicular (long arrow) steatosis (H&E, ×200 magnification). Patient 4 underwent LT at age 1 year and 4 months. (F) The explanted liver demonstrated cirrhosis (inset: H&E, ×200 magnification) and contained a well-differentiated HCC (main image: H&E, ×200 magnification).

damaging, a single mutation in bile salt export pump (BSEP) would not explain the patient's phenotype. Subsequent WES analysis revealed a homozygous nonsense mutation in *TALDO1*: c.412C>T; p.(Arg138Ter). Sanger sequencing confirmed homozygosity in the patient and heterozygosity in the parents.

PATIENT 2

Patient 2 (female) presented at the age of 5 months with vomiting and hepatosplenomegaly. The diagnosis of gastro-esophageal reflux was made. She was the first child of parents of respective Turkish and British ancestry.

Laboratory assessment, including liver biochemistry, was unremarkable (Table 1). Liver USS and magnetic resonance imaging (MRI) showed multiple liver lesions on the background of a mildly fatty liver and splenomegaly. Liver histology at the age of 9 months showed nodularity and mild macrovesicular steatosis with no evidence of cholestasis or inflammation (Fig. 1).

Work-up included normal respiratory chain enzymes on muscle biopsy. Filipin staining for Niemann-Pick disease type C on skin fibroblasts was negative, and no pathogenic variants in mitochondrial DNA genes (*POLG* [DNA polymerase gamma, catalytic subunit], *MPV17* [mitochondrial inner membrane protein], *DGUOK* [deoxyguanosine kinase], and *TRMU* [TRNA mitochondrial 2-thiouridylase]) were identified. Genetic testing revealed compound heterozygosity for variants c.542C>T, p.(Ala181Val) and c.560C>T, p.(Ser187Phe) (Table 1) in *TALDO1*, both occurring at highly conserved amino acids and predicted to be deleterious. Parental sequencing confirmed compound heterozygosity. Diagnosis was strongly supported by skin fibroblast enzyme activity of 1 nmol/mg protein (normal range: 14-24).[1] Follow-up MRI confirmed hepatomegaly with an irregular nodular outline suggestive of cirrhosis, and numerous hepatic nodules. The nodules showed contrast enhancement features in keeping with dysplastic/regenerative nodules.

Echocardiography confirmed a structurally and functionally normal heart.

At last follow-up, at age 5 years, she was on the 67th and 87th centiles for height and weight, respectively. Although at follow-up liver radiological appearances have not demonstrated further progression and her AFP is normal, the risk of developing HCC remains a concern. Liver function tests remain normal, as is her neurodevelopment.

PATIENT 3

Patient 3 (female) presented at the age of 3 months with conjugated hyperbilirubinemia, coagulopathy, failure to thrive, hepatosplenomegaly, and abdominal distension (Table 1). She was the fourth child of consanguineous Pakistani parents. In the immediate postnatal period, she suffered from recurrent hypoglycemia and hypothermia, which resolved. As part of her work-up, an abdominal USS and CT scan were performed that showed splenomegaly and a right liver lobe hemangiomatous lesion.

She followed 0.4th centile for weight, first centile for length, and <0.4th centile for head circumference. Gross motor developmental delay and possible proximal muscle weakness were noted, but brain MRI scan and electroencephalogram were normal. Mitochondrial DNA depletion studies were negative. At the age of 5 months, she underwent LT for subacute liver failure. The explanted liver showed cirrhosis and both microvesicular and macrovesicular steatosis with abundant canalicular cholestasis. No focal changes were found, apart from the previously noted small hemangioma.

Five years after LT, due to calcineurin inhibitor–induced renal dysfunction, she was converted to mycophenolate mofetil and low-dose maintenance prednisolone. At the age of 16 years, she was diagnosed with premature ovarian insufficiency, leading to pubertal delay and short stature; hormone replacement therapy was started.

As no unifying diagnosis for her multisystem condition was reached, she had cholestasis gene panel testing, which confirmed homozygosity for c.695_696del p.(Ile232fs) in *TALDO1*. Skin fibroblast enzyme activity was significantly reduced at 1 nmol/mg protein.[1]

At her most recent follow-up, 21 years after LT, she has normal liver graft function and a normal echocardiogram. She has mild learning difficulties but attends mainstream education.

PATIENT 4

Much of the case history of patient 4 (female) was described previously (patient 3 in Al-Shamsi

et al.[12]); here we highlight additional features of the case. She was transferred to King's College Hospital with coagulopathy, conjugated hyperbilirubinemia with normal gamma-glutamyltransferase (GGT), and elevated AFP (Table 1). Abdominal USS and axial imaging showed a homogeneous liver, splenomegaly, and three focal liver lesions (largest up to 3.2 cm). She underwent a living-related LT for acute-on-chronic liver failure, from her mother at 17 months of age.

The explanted liver showed cirrhotic changes, a single well-differentiated HCC, and multiple regenerative nodules. Immunostaining for BSEP, MDR3, TJP2, GGT, clusters of differentiation 13 (CD13), claudin-1, and CD66 was preserved. After LT, she developed recurrent chest infections and wheeze.

Persistent systolic hypertension prompted a renal biopsy, which showed nonspecific findings with mild mesangial hypercellularity. Early-onset liver failure, wrinkly dry skin, and biochemical confirmation of abnormal urine polyols raised the possibility of transaldolase deficiency. Subsequent genetic analysis demonstrated homozygosity for c.574C>T, p.(Arg192Cys) mutation in TALDO1.[12] At the age of 10 years, she has normal liver graft function. She has moderate failure to thrive and systemic hypertension controlled by enalapril with normal glomerular filtration rate (73 mL/min/1.73 m^2). She has a normal neurodevelopment.

Discussion

We report here 4 children with transaldolase deficiency presenting with liver involvement, including early-onset cirrhosis and HCC.

A biochemical diagnosis of transaldolase deficiency is only possible in a small number of laboratories.[7] On the other hand, the clinical use of NGS, including genetic panels with TALDO1 or WES, has led to the easier identification of new cases.

Previous publications have shown that transaldolase deficiency affects several body systems. In the liver this has manifested with both cirrhosis and liver failure. Other features can include involvement of skin and lungs, structural and functional cardiac defects, fetal hydrops, anemia and pancytopenia, soft dysmorphic features, and a spectrum of renal abnormalities.[4] In addition to liver disease, our patients variously manifest renal, cardiac, endocrine, and neurodevelopmental

involvement. Close monitoring for these features in patients with transaldolase deficiency is highly recommended, regardless of LT. LT is curative of the liver disease but clinicians need to be vigilant of possible other system involvement.

Two of the 3 transplanted patients in our series had HCC in the explanted livers; 1 patient with transaldolase deficiency and HCC had previously been described.[13] In the 2 patients, HCC was diagnosed before the age of 18 months. Of our 2 patients without HCC, 1 was transplanted at only 5 months of age, whereas in the other, the liver had focal dysplastic nodules at presentation and a very careful surveillance is in place. HCC has been described in almost all chronic liver diseases but it still remains very rare in early childhood. There have been isolated reports of radiological nodular changes in the liver of patients with transaldolase deficiency.[12,14] The diseases associated with early presentation of HCC are tyrosinemia type 1 and BSEP deficiency.[15,16] Transaldolase deficiency should now also be considered as a cause of early-onset HCC. In the murine model of transaldolase deficiency, 46% of knockout mice develop HCC. Strikingly, the heterozygous mice also develop HCC much more frequently than wild-type mice.[17] Liver carcinogenesis was associated with oxidative stress due to overexpression of genes such as c-Jun, reduced phosphorylation of β-catenin, and inhibition of Fas apoptosis.[17] N-acetylcysteine has been shown to have a protective effect on the hepatocytes mainly through normalization of the β-catenin phosphorylation process in the same animal model.

Transaldolase deficiency remains a rare cause of early-onset normal GGT liver disease. The wider use of genetic testing does mean that patients are now likely to be diagnosed much earlier in their course. The prospective management of such patients should consider the multisystem nature of the disease, meaning that LT could have increased risks. The native liver (due to unknown mechanisms) remains at lifelong risk of malignant transformation. Additionally, transaldolase deficiency should be excluded in children presenting with HCC on the background of unexplained liver disease.

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