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ORIGINAL ARTICLE

Genotype correlates with clinical course and outcome of children with tight junction protein 2 (TJP2) deficiency–related cholestasis

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Abbreviations: ACMG, American College of Medical Genetics and Genomics; AFP, alfa-fetoprotein; cPELD, calculated pediatric end-stage liver disease score; LT, liver transplantation; NLS, native liver survival; OS, overall survival; PFIC, progressive familial intrahepatic cholestasis; PIBD, partial internal biliary diversion; PILBD, paucity of interlobular bile ducts; PPTM, predicted protein-truncating mutations; sBA, serum bile acids; SBD, surgical biliary diversion; SIFT, sorting intolerant from tolerant; TJP2, tight junction protein 2.

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Abstract

Background and Aims: The study aimed to describe the clinical course and outcomes, and analyze the genotype-phenotype correlation in patients with tight junction protein 2 (TJP2) deficiency.

Approach and Results: Data from all children with chronic cholestasis and either homozygous or compound heterozygous mutations in *TJP2* were extracted and analyzed. The patients were categorized into 3 genotypes: *TJP2-A* (missense mutations on both alleles), *TJP2-B* (missense mutation on one allele and a predicted protein-truncating mutation [PPTM] on the other), and *TJP2-C* (PPTMs on both alleles). A total of 278 cases of genetic intrahepatic cholestasis were studied, with TJP2 deficiency accounting for 44 cases (15.8%). Of these, 29 were homozygous and 15 were compound heterozygous variants of *TJP2*. TJP2-A genotype was identified in 21 (47.7%), TJP2-B in 7 cases (15.9%), and TJP2-C in 16 cases (36.4%), respectively. Patients with the TJP2-C genotype were more likely to experience early infantile cholestasis (87.5% vs. 53.5%, $p = 0.033$), less likely to clear jaundice (12.5% vs. 52.2%, $p = 0.037$), more likely to develop ascites, and had higher serum bile acids. Patients with the TJP2-C genotype were more likely to die or require liver transplantation (native liver survival: 12.5% vs. 78.6%, $p < 0.001$), with a median age at death/liver transplantation of 2.5 years. Cox regression analysis revealed that TJP2-C mutations ($p = 0.003$) and failure to resolve jaundice ($p = 0.049$) were independent predictors of poor outcomes.

Conclusions: Patients with the TJP2-C genotype carrying PPTMs in both alleles had a rapidly progressive course, leading to early decompensation and death if they did not receive timely liver transplantation.

INTRODUCTION

Tight junction protein 2 (TJP2), also known as zona occludens 2, is a cytoplasmic protein that acts as a scaffold connecting the transmembrane proteins (claudins) to the actin cytoskeleton required to maintain the blood-bile barrier, thus protecting hepatocytes.[1,2] Hepatocytes of patients carrying variants in *TJP2* demonstrate intracellular inclusions of disrupted apical membrane structures, altered canalicular transporters, and disrupted cellular polarity due to the disruption of the blood-bile barrier and the leakage of biliary components into hepatocytes through paracellular spaces.[1,3] With the advent of genetic testing, TJP2 deficiency is being increasingly diagnosed and accounts for 12%–23% of children presenting with a phenotype of progressive familial intrahepatic cholestasis (PFIC).[4,5] There are wide variations in phenotypic presentation and severity, with early onset disease, progressive course, and poor outcomes reported in children with predicted protein-truncating mutations (PPTMs).[6–8] In contrast, adult-onset cholestasis and intrahepatic cholestasis of pregnancy have been reported in some cases of TJP2 deficiency, especially in those with heterozygous mutations.[9–11] TJP2 is a component of tight junctions in various epithelial cells, which may explain the myriad extrahepatic manifestations, such as sensorineural deafness, renal tubular dysfunction, hypomyelination, respiratory issues, and myopia reported in patients with TJP2 deficiency.[6,12–14] Another peculiar finding is the early development of HCC in these children, which is often detected on routine imaging or in explant specimens.[12,15,16]

There are no large studies describing the natural course, genotype-phenotype correlation, and outcomes of patients with TJP2 deficiency. The present study aimed to describe the clinical course, outcomes, and genotype-phenotype correlation in patients with chronic cholestasis due to TJP2 deficiency. This study was part of a multicenter national registry of patients with PFIC.

METHODS

The present study was a retrospective analysis of data collected from a national multicenter registry of patients with PFIC in India. All data in the registry were collected in accordance with the 2013 Declaration of Helsinki and the 2018 Declaration of Istanbul after obtaining ethical approval from the respective institutional ethics committees. As this was a retrospective study, a waiver of consent was obtained from the respective ethics committees. The data were collected in a prespecified format in Microsoft Excel and shared by each center. Data from all children (0–18 y of age) presenting with chronic cholestasis and harboring either homozygous or compound heterozygous mutations in *TJP2* were extracted and analyzed. Genetic analysis was performed at the respective centers using next-generation sequencing, either as a multigene cholestasis panel or whole-exome sequencing. The American College of Medical Genetics and Genomics (ACMG) criteria were used to report and define the pathogenicity of the identified mutations. Patients whose genetic reports were not shared by collaborators were excluded. Patients who simultaneously harbored homozygous mutations in other genes were also excluded. For uniformity, all variants described in this manuscript were annotated based on the Ensembl transcript ENST00000539225 (NM _001170416). In silico prediction tools (Mutation Taster 2, CADD [Combined Annotation Dependent Depletion] score, SIFT [sorting intolerant from tolerant], and PolyPhen-2 [HumDiv]) were used to determine the predicted effects of the variants. Patients with variants reported as variants of uncertain significance were excluded if they were consistently predicted to be benign or likely benign by the majority of the in silico prediction tools. All in silico computations were run at the host center based on chromosomal annotation, Ensembl transcript ID, position of the variation, and version of the build of the genome, as provided in the copy of the genetic reports shared by the collaborators. All variants were assessed using Mutation Taster 2 (2021, freely available online), whereas single nucleotide variations were additionally assessed using the CADD score (GRCh38-v1.6/GRCh37-v1.6) (freely available online: https://cadd.gs.washington.edu/snv). Polyphen and SIFT categorizations were performed for all the missense variants.

We further categorized each patient as harboring 1 of the 3 following genotypes: *TJP2-A* (carrying missense mutations on both alleles), *TJP2-B* (carrying missense mutation on one allele and PPTM on the other), and *TJP2-C* (carrying PPTMs on both alleles). This classification was developed based on observations from a case series in which patients with biallelic PPTMs had rapidly progressive disease and poor outcomes.[6,7] Clinical, biochemical, histopathological, endoscopic, and outcome-related data were collected at each center by the respective investigators and relayed to the host center. Infantile cholestasis was defined as the presence of conjugated hyperbilirubinemia (direct bilirubin >1 mg/dL).[17] Early infantile cholestasis was defined as the onset of cholestasis within the first 3 months of life. Pruritus was graded according to the clinical scratch scale as follows: grade 1, pruritus only when undistracted; grade 2, pruritus not affecting sleep or daily activities; grade 3, pruritus affecting sleep or daily activities; and grade 4, severe pruritus resulting in cutaneous abrasions or mutilations. All relevant clinical details, including surgical interventions, such as surgical biliary diversion (SBD) or liver transplantation (LT), were included. The type of biliary diversion (partial internal biliary diversion [PIBD] or partial external biliary diversion) was noted and clinical and biochemical data were collected before and at the last follow-up after SBD. Liver histopathology findings were collected under one or more of the following subheadings: (i) bland cholestasis, (ii) predominant giant cell transformation, (iii) paucity of interlobular bile duct (PILBD)—interlobular bile duct to portal tract ratio of <0.6 in a liver biopsy specimen containing at least 6 portal tracts, and (iv) biliary obstruction, including ductular proliferation and bile plugs. Immunostaining of liver histopathology was performed at a few centers; however, none of the centers performed TJP2 or claudin 1 immunostaining. The amount of fibrosis on liver biopsy was graded according to the METAVIR score.[18] Liver histopathological specimens from explants and postmortem biopsies were not included in the analysis to compare histopathological findings across genotypes. All biochemical parameters were converted to standard units at the reporting centers. A calculated pediatric end-stage liver disease (cPELD) score was derived for each patient irrespective of age using the formula: cPELD = 4.80 (Ln serum bilirubin [mg/dL]) + 18.57 (Ln INR)−6.87 (Ln albumin [g/dL])+ 4.36 (<1-year-old) + 6.67 (growth failure).[19] The presence of splenomegaly with an increased portal vein diameter (according to an age-specific cutoff) with or without hepatofugal flow in the main portal vein was considered as sonographic evidence of portal hypertension. Patients at all centers underwent contrast-enhanced CT of the abdomen if their alfa-fetoprotein levels were elevated above age-specific norms. Risk stratification of varices was performed as high-risk (grade 3 varices or grade 2 varices with red color signs) and low-risk (grade 2 varices without red color sign or grade 1 varices).[20] Hepatopulmonary syndrome was defined based on the alveolar-arterial oxygen gradient on arterial blood gas and the presence of an intrapulmonary shunt on saline contrast echocardiography.[21] Minor differences were observed in the medical management protocols at various centers. Ursodeoxycholic acid and fat-soluble vitamin supplements were consistently used in all centers. In most centers, ursodeoxycholic acid is the first-line drug for pruritus management and rifampicin is

the preferred second-line agent. Outcome variables included native liver survival (NLS), overall survival (OS), death, and LT. OS included survival with the native liver or graft liver. The age at death or LT was recorded.

Statistical analysis

Continuous variables are expressed as median and IQR, and categorical values are expressed as proportions. The Mann-Whitney *U* test was used to compare continuous variables, and the Fisher exact test was used to compare categorical variables. All statistical analyses were performed using SPSS version 22. Kaplan-Meier survival analysis was used to compare and depict NLS and OS among patients with various genotype categories. Log-rank tests with pairwise comparisons were used to identify statistically significant differences in outcomes. Univariate analysis was first used to identify predictors of poor outcomes, which were then entered into the Cox regression model to identify independent predictors of poor outcomes. We also conducted a thorough literature review and collated the data of all children with genetically confirmed TJP2 deficiency–related cholestasis from the published literature (criteria for inclusion: a minimum of 2 children reported in the case series). We then analyzed the outcomes, genotype-phenotype correlation, and the incidence of HCC in the entire cohort (including our patients and those reported in the literature).

RESULTS

A total of 281 patients with homozygous or compound heterozygous variants in the genes responsible for genetic intrahepatic cholestasis were enrolled in the National Multicenter Registry (*ABCB11* [86], *ABCB4* [64], *TJP2* [47], *ATP8B1* [42], *USP53* [17], *MYO5B* [11], *NR1H4* [8], KIF12 [4], LSR [1], and VPS33B [1]). Variants in TJP2 were reported in 47 cases, of which 3 were excluded. One child with homozygous and another with compound heterozygous missense variants in *TJP2* were excluded from the analysis because the variants detected were found to be likely benign using in silico prediction tools (Mutation Taster, SIFT, Polyphen, and CADD). The first child also harbored homozygous variants of *GPD1* and did not exhibit the clinical phenotype of chronic cholestasis. One child was excluded because the collaborating institute could not share the genetic report. Thus, TJP2 deficiency accounted for 44/278 (15.8%) cases of genetic intrahepatic cholestasis in the registry. Of these, 29 children were homozygous, and 15 harbored compound heterozygous variants in *TJP2*. These 44 cases were reported in 39 families. Four families had more than 1 affected member. In addition, there was a family history of end-stage cholestatic liver disease (without genetic diagnosis)-related death at the age of 2 years in a cousin of one of the patients. A history of intrahepatic cholestasis of pregnancy was present in 6 (15.4%) of 39 mothers of children with TJP2 deficiency–related disease during 1 or more pregnancies. Two patients with compound heterozygous variants in *TJP2* also harbored an additional heterozygous variant in *CFTR* and *KIF12*. Fifteen (34.1%) of these children were born to consanguineous, third-degree parents. The median age at enrollment was 57 months (IQR: 15.5–102.5) while the median age at the last follow-up was 64 months (IQR: 20–103) for the surviving patients. The age at death was considered as the age at enrollment for patients who died before enrollment in the registry. The TJP2-A genotype was identified in 21 (47.7%), TJP2-B in 7 (15.9%), and TJP2-C in 16 (36.4%) patients. One patient with 3 different heterozygous variants (2 in 1 allele and 1 in another, based on parental sequencing), 2 of which were PPTMs and 1 was a missense variant, was classified as TJP2-C.

Clinical presentation and natural course

Jaundice was the initial presenting symptom in 40 patients (90.9%), with a median age at onset of 2 months (IQR: 1–6 mo). Early onset (first 3 mo) infantile cholestasis was present in 29 (65.9%) patients. Pruritus developed in 34 patients (77.3%) at a median age of 8 months (IQR: 4.3–12 mo). Twenty-two (64.7%) patients had significant pruritus (clinical scratch scale ≥ 3). Three (8.8%) patients underwent SBD for refractory pruritus, with successful resolution in 2 (66.7%) patients. Table 1 shows the demographic, clinical, and biochemical characteristics of the entire cohort ($n = 44$). Five (11.4%) patients had gallstones. Nine extrahepatic manifestations were observed in 7 patients, the most common being recurrent diarrhea ($n = 4$) and pancreatitis ($n = 2$). Both patients with pancreatitis had gallstones. Growth failure was observed in 54.5% of the patients at the initial presentation. Liver histopathology was available for 37 of the 44 patients. Five patients with only explant histopathology and 1 patient with only postmortem biopsy were excluded. The remaining 31 histopathological samples were obtained through liver biopsy at a median age of 12 months (IQR: 7–19). Seven of these patients also had additional explant histopathology. Among patients who underwent percutaneous liver biopsy, the findings were variable: prominent portal and lobular inflammation with giant cell transformation (29%), PILBD (29%), and bland cholestasis with no or minimal inflammation (25.8%). Thirteen patients (42%) had advanced fibrosis (F3-F4

TABLE 1 Demographic, clinical, biochemical, histopathological, endoscopic, and outcome data of children with chronic cholestasis due to deficiency of TJP2

Parameter	TJP2 deficiency (n = 44)
Gender, n (%)	
Male	25 (56.8)
Female	19 (43.2)
Mutation type, n (%)	
Homozygous	29 (65.9)
Compound heterozygous	15 (34.1)
Categorization of genotype, n (%)	
TJP2-A (Missense mutations on both alleles)	21 (47.7)
TJP2-B (PPTM on 1 allele)	7 (15.9)
TJP2-C (PPTMs on both alleles)	16 (36.4)
Clinical presentation, n (%)	
Jaundice	40 (90.9)
Age of onset of jaundice (mo)	2 (1–6)
Early onset of cholestasis (first 3 mo)	29 (65.9)
Pruritus	34 (77.3)
Age at onset (mo)	8 (4.3–12)
Grade 3 or 4 pruritus	22/34 (64.7)
Pruritus requiring SBD	3/34 (8.8)
Growth failure	24 (54.5)
Extrahepatic manifestations, n (%)	7 patients (9 manifestations)
Recurrent diarrhea	4 (9.1)
Recurrent respiratory illnesses	2 (4.5)
Pancreatitis	2 (4.5)
Seizures	1 (2.3)
Sensorineural hearing loss	0
Myopia	0
Liver histopathological findings (excluding explants and postmortem biopsy), n (%)	Available in 31
Predominant findings	
Bland cholestasis	8 (25.8)
Giant cell transformation	9 (29)
PILBD	9 (29)
Fibrosis as per METAVIR	
F1-F2	18 (58)
F3-F4 (advanced fibrosis)	13 (42)
Biochemical variables	
Bilirubin at presentation (mg/dL)	8.1 (1.1–15)
Alanine aminotransferase (IU/L)	70.5 (47–235.3)
Serum albumin (g/dL)	3.9 (3.3–4.2)
INR	1.1 (1–1.4)
Serum bile acids (μmol/L)	176 (90.1–255.2)
Calculated PELD	15 (7–21)
Complications, n (%)	
Ascites	15 (34.1)
Age at the development of ascites (mo)	24 (10.5–78)
HE	3 (6.8)
Variceal bleed	6 (13.6)
Hepatopulmonary syndrome	4/23 (17.4)
Outcome (at last follow-up), n (%)	
Native liver survival	24 (54.5)
Death	7 (15.9)
Liver transplantation	13 (29.5)
Healthy graft	11/13 (84.6)
Retransplant/death	2/13 (15.4)

Note: Continuous variables are expressed as median (IQR) and categorical values as proportions.
Abbreviations: PELD, pediatric end-stage liver disease; PILBD, paucity of interlobular bile ducts; PPTM, predicted protein-truncating mutation; SBD, surgical biliary diversion; TJP2, tight junction protein 2.

METAVIR). All 7 patients with paired histopathology (initial biopsy followed by explant) showed progression of fibrosis with an evidence of cirrhosis at the explant. Serum bile acid (sBA) levels were elevated in all patients, with median levels of 176 μmol/L (IQR: 90.1–255.2). The median gamma-glutamyl transpeptidase was 41 IU/L (IQR: 25.5–70.5). Ascites developed in 15 patients (34.1%) at a median age of 24 months (IQR: 10.5–78). At the time of the last recorded follow-up, 24 (54.5%) patients survived with their native liver at a median age of 60 months (IQR: 22–103). Thirteen (29.5%) patients underwent LT. Seven (15.9%) patients died because they did not have a related donor or decided against LT. The median age at death/LT was 4.5 years (IQR: 2–8 y). None of the included patients had HCC on imaging or explant specimens.

Genotype-phenotype correlation

The details of the genetic variations, variant classifications, in silico prediction results, and genotype categorization are described in Table 2. The clinical course and outcomes were compared according to the genotype categorization: TJP2-A (21 patients), TJP2-B (7 patients), and TJP2-C (16 patients). As shown in Table 3, patients with the TJP2-C genotype (carrying PPTMs on both alleles) were more likely to present with early infantile cholestasis (87.5% vs. 53.5%, $p = 0.033$) and had a lower probability of jaundice resolution on medical therapy (12.5% vs. 52.2%, $p = 0.037$). Although there was no difference in the incidence of pruritus or its age of onset, pruritus was less likely to resolve with medical therapy in patients with TJP2-C than in others (30.8% vs. 71.4%,

TABLE 2 Details of the genetic variations, variant classification, in silico prediction results, genotype classification, and outcomes of patients with TJP2 deficiency

Patient no.	Family no.	TJP2: variant 1 (Original variant classification)	In silico prediction results[a]	TJP2: Variant 2 (Original variant classification)	In silico prediction results[a]	Genotype category assigned	Outcome
1	1	c.1571_1595dup (p. Asp533AsnfsTer6) [P]	MT2: Deleterious (NMD)	c.1571_1595dup (p. Asp533AsnfsTer6) [P]	MT2: Deleterious (NMD)	TJP2-C	LT at 18 mo for end-stage liver disease
2	2	c.2602C > T (p. Arg868Ter) [P]	MT2: Deleterious (NMD) CADD: 44	c.2602C > T (p. Arg868Ter) [P]	MT2: Deleterious (NMD) CADD: 44	TJP2-C	LT at 26 mo for end-stage liver disease
3	3	c.2988delC (p. Ser997AlafsTer8) [P]	MT2: Deleterious (NMD)	c.2988delC (p. Ser997AlafsTer8) [P]	MT2: Deleterious (NMD)	TJP2-C	Death at 4.5 y due to end-stage liver disease while on the waitlist
4	4	c.3418G > T (p. Glu1140Ter) [VUS]	MT2: Deleterious (NMD) CADD: 45	c.2459_2462dupCACT (p. Leu822ThrfsTer52) [LP]	MT2: Deleterious (NMD)	TJP2-C	Another variant c.215A > C (p.Lys72Thr) [VUS] (CADD- 26.5) detected; LT at 5 y for refractory pruritus and growth failure
5	5	c.1613+1G > A [LP]	MT2: Deleterious (splice site lost) CADD: 34	c.1613+1G > A [LP]	MT2: Deleterious (splice site lost) CADD: 34	TJP2-C	Death at 12 mo of age due to end-stage liver disease
6	6	c.3319A > T (p. Lys1107Ter) [P]	MT2: Deleterious (NMD) CADD: 43	c.3319A > T (p. Lys1107Ter) [P]	MT2: Deleterious (NMD) CADD: 43	TJP2-C	Death at 2.5 y due to end-stage liver disease
7		c.3319A > T (p. Lys1107Ter) [P]	MT2: Deleterious (NMD) CADD: 43	c.3319A > T (p. Lys1107Ter) [P]	MT2: Deleterious (NMD) CADD: 43	TJP2-C	Death due to end-stage liver disease at 2 y
8	7	c.2473delA (p. Thr825Leufs[a]8) [P]	MT2: Deleterious (NMD)	c.2473delA (p. Thr825Leufs[a]8) [P]	MT2: Deleterious (NMD)	TJP2-C	PEBD at 10 mo, partial relief after PEBD LT at 2 y, death in immediate post-LT period
9	8	c.1614-11_1620del (3' Splice variant) [P]	MT2: Deleterious (NMD)	c.1614-11_1620del (3' Splice variant) [P]	MT2: Deleterious (NMD)	TJP2-C	Death at 12 mo of age due to end-stage liver disease
10	9	c.459delC (p. Gln154ArgfsTer20) [LP]	MT2: Deleterious (NMD)	c.459delC (p. Gln154ArgfsTer20) [LP]	MT2: Deleterious (NMD)	TJP2-C	LT at 7 y of age due to end-stage liver disease
11	10	c.1614- 3_1614-2insATA [LP]	MT2: Deleterious (NMD)	c.561_562insGA (p.ser190GlyfsTer4) [LP]	MT2: Deleterious	TJP2-C	LT at 9 mo due to progressive jaundice and decompensation (ascites)
12	11	c.1765-1G > C (3′ splice site) [P]	MT2: Deleterious	c.1765-1G > C (3′ splice site) [P]	MT2: Deleterious	TJP2-C	LT at 5 y of age due to end-stage liver disease
13	12	c.714_773del (p. Gln238_Asp257-del) [LP]	MT2: Deleterious	c.714_773del (p. Gln238_Asp257del) [LP]	MT2: Deleterious	TJP2-C	LT at 12 mo for end-stage liver disease
14	13	c.2473delA p.Thr825Leufs[a]8 [P]	MT2: Deleterious (NMD)	c.2473delA p.Thr825Leufs[a]8 [P]	MT2: Deleterious (NMD)	TJP2-C	LT at 12 mo due to progressive jaundice and portal hypertension

15	14	c.435+1_435+2del [LP]	MT2: Deleterious (Splice site changes)	c.2096dup (p. Met699IlefsTer53) [LP]	MT2: Deleterious (NMD)	TJP2-C	Listed for LT for progressive jaundice and refractory pruritus; surviving with native liver at 18 mo
16	15	c.3418G > T (p. Glu1140Ter) [LP]	MT2: Deleterious (NMD) CADD: 45	c.3418G > T (p. Glu1140Ter) [LP]	MT2: Deleterious (NMD) CADD: 45	TJP2-C	PIBD at 3 y for refractory pruritus; surviving with native liver at 14 y
17	16	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	CNV deletion Exon 1 to 16 [LP]	In silico prediction not available	TJP2-B	LT at 15 y of age due to end-stage liver disease
18	17	c.2602C > T (p. Arg868Ter) [P]	MT2: Deleterious (NMD) CADD: 44	c.389C > T (p. Ser130Leu) [VUS]	MT2: Deleterious PP2: Possibly damaging SIFT: Deleterious CADD: 24.9	TJP2-B	LT at 3 y for progressive jaundice and growth failure; death after uncontrolled sepsis
19	18	Intron 1 c.60+1G > C (5′ splice site) [LP]	MT2: Deleterious CADD: 33	c.1068T > A (p. Ser356Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 23.8	TJP2-B	Surviving with the native liver at 14 mo
20	19	Intron 6 c.1149 +1G > A (splice site) [P]	MT2: Deleterious (NMD)	c.2333T > C (p. Leu778Ser) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.3	TJP2-B	Surviving with the native liver at 3.5 y
21	20	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	c.940C > T (p. Arg314Ter) [LP]	MT2: Deleterious (NMD) CADD: 35	TJP2-B	Surviving with the native liver at 6.5 y
22	21	c.683_694del (p. Arg226_Gly229del)	MT2: Deleterious	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-B	Surviving with the native liver at 18 mo
23	22	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	c.1345G > T (p. Glu449Ter) [LP]	MT2: Deleterious (NMD) CADD: 43	TJP2-B	Surviving with the native liver at 2 y
24	23	c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	TJP2-A	Death due to end-stage liver disease at 18 y of age
25		c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	TJP2-A	LT at 5.5 y for refractory pruritus and growth failure
26		c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	TJP2-A	LT at 15 y for decompensated cirrhosis and intractable pruritus
27	24					TJP2-A	Surviving with the native liver at 20 mo

TABLE 2. (*continued*)

Patient no.	Family no.	TJP2: variant 1 (Original variant classification)	In silico prediction results[a]	TJP2: Variant 2 (Original variant classification)	In silico prediction results[a]	Genotype category assigned	Outcome
		c.2288C > G; (p. Pro763Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 30	c.1058G > A (p. Arg353Gln) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32		
28	25	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	TJP2-A	Died at 8 y while on the waitlist
29	26	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	c.2938A > C (p. Ile980Leu) [VUS]	MT2: Benign PP2: Possibly damaging SIFT: Tolerant CADD: 22.5	TJP2-A	Surviving with the native liver at 5 y
30	27	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	c.1687G > A (p. Gly563Arg) [LP]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	TJP2-A	Surviving with the native liver at 7 mo
31	28	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 5.5 y
32		c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	PIBD at 2 y for refractory pruritus; surviving with the native liver at 8.5 y
33	29	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 13.5 y
34	30	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 6 mo
35	31	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 10 y
36	32	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 2.5 y

37	33	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 9 y
38	34	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 7 mo
39		c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	c.2465T > C (p. Leu822Pro) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.7	TJP2-A	Surviving with the native liver at 6 y
40	35	c.1351C > T (p. Arg451Cys) [VUS]	MT2: Benign PP2: Probably damaging SIFT: Deleterious CADD: 29.1	c.1058G > A (p. Arg353Gln) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	TJP2-A	Surviving with the native liver at 10 y
41	36	c.1676G > A (p. Gly559Asp) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 29.7	c.1676G > A (p. Gly559Asp) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 29.7	TJP2-A	Surviving with the native liver at 14 y
42	37	c.1068T > A (p. Ser356Arg) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 23.8	c.2333T > C (p. Leu778Ser) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 28.3	TJP2-A	Surviving with the native liver at 5.5 y
43	38	c.317C > T (p. Ala106Val) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 27.5	c.317C > T (p. Ala106Val) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 27.5	TJP2-A	Surviving with the native liver at 7 y
44	39	c.389C > T (p. Ser130Leu) [VUS]	MT2: Deleterious PP2: Possibly damaging SIFT: Deleterious CADD: 24.9	c.1058G > A (p. Arg353Gln) [VUS]	MT2: Deleterious PP2: Probably damaging SIFT: Deleterious CADD: 32	TJP2-A	Surviving with the native liver at 2 y

Note: All variants described in the manuscript have been annotated based on Ensembl transcript ENST00000539225 (NM _001170416) for uniformity. Categorization of TJP2 genotype: TJP2-A: carrying missense mutations on both the alleles; TJP2-B: carrying missense mutation on 1 allele and PPTM on the other; TJP2-C: carrying PPTMs on both the alleles.

[a]In silico prediction tools: MT2: Mutation Taster 2 (2021); PP2: PolyPhen-2; SIFT: Sorting Intolerant from tolerant prediction algorithm; CADD: Combined Annotation Dependent Depletion score (GRCh38-v1.6/ GRCh37-v1.6).

Abbreviations: LP, likely pathogenic variant; LT, liver transplantation; NMD, nonsense mediated mRNA decay; P, pathogenic variant; PEBD, partial external biliary diversion; PIBD, partial internal biliary diversion; PPTM, predicted protein-truncating mutations; TJP2, tight junction protein 2; VUS, variant of uncertain significance.

TABLE 3 Comparison of outcomes and clinical, biochemical, and histopathological parameters of children with biallelic predicted protein-truncating mutation (TJP2-C) and those without biallelic predicted protein-truncating mutations (TJP2-A and TJP2-B) in TJP2 deficiency

Parameter	TJP2-A and TJP2-B (n = 28)	TJP2-C (n = 16)	Significance (nonparametric) *p*
Jaundice	23 (82.1)	16 (100)	0.139
Age at the onset of jaundice (mo)	3 (2–6)	1.3 (1–3)	0.061
Early onset of cholestasis (first 3 mo)	*15* (*53.5*)	*14* (*87.5*)	*0.033*
Resolution of jaundice	*12/23* (*52.2*)	*2* (*12.5*)	*0.037*
Pruritus	21 (75)	13 (81.3)	0.719
Age of onset in pruritus (mo)	7 (4.5–20.5)	9.5 (4.3–12)	0.985
Resolution of pruritus	*15/21* (*71.4*)	*4/13* (*30.8*)	*0.033*
Growth failure	16 (57.1)	8 (50)	0.761
Ascites	*5* (*17.9*)	*10* (*62.5*)	*0.043*
Age at onset of ascites (mo)	*96* (*54–156*)	*18* (*6.5–24*)	*0.002*
Portal hypertension on ultrasound	*11/25* (*44*)	*10/12* (*83.3*)	*0.032*
Hepatopulmonary syndrome	1/19 (5.2)	3/14 (21.4)	0.288
Significant fibrosis (F3-F4 METAVIR)	6/21 (28.6)	7/10 (70)	0.052
Peak bilirubin (mg/dL)	*7.5* (*2–11.2*)	*17* (*14.5–20.4*)	*< 0.001*
Peak INR	1.4 (1.03–1.6)	1.5 (1.15–2.1)	0.558
Alanine aminotransferase (IU/L)	*60* (*36–135.5*)	*143* (*67–412*)	*0.02*
Albumin (g/dL)	*3.9* (*3.54–4.3*)	*3.4* (*2.8–4*)	*0.058*
Serum bile acids (μmol/L)	*139* (*90–208*)	*231.5* (*165–316*)	*0.026*
Calculated PELD score	*8.5* (*3.8–18.1*)	*16.8* (*13.6–21.8*)	*0.019*
Alfa-fetoprotein (ng/mL)	56 (2.1–2744)	65.5 (16.9–542.5)	0.711
Outcome			
Native liver survival	*22* (*78.6*)	*2* (*12.5*)	*< 0.001*
Liver transplantation	*4* (*14.3*)	*9* (*56.2*)	
Death on the waitlist	*2* (*7.1*)	*5* (*31.3*)	
Age at death/liver transplantation (y)	*9* (*4.5–15.8*)	*2.5* (*2–5*)	*0.009*

Note: Continuous variables are expressed as median (IQR) and categorical values as proportions; Mann-Whitney *U* test was used to compare the continuous variables, and the Fisher exact test to compare the categorical variables. Categorization of TJP2 genotype: TJP2-A: carrying missense mutations on both the alleles; TJP2-B: carrying missense mutation on 1 allele and PPTM on the other; TJP2-C: carrying PPTMs on both the alleles.
Italic values indicates are statistically significant.
Abbreviations: PELD, pediatric end-stage liver disease score; PPTM, predicted protein-truncating mutation; TJP2, tight junction protein 2.

$p = 0.033$). Ascites developed more commonly and rapidly in the TJP2-C genotype. Patients with TJP2-C developed ascites at a median age of 18 months (IQR: 6.5–24). Biochemical markers of liver disease severity (bilirubin and sBA) were significantly higher in the TJP2-C group than in the TJP2-A and TJP2-B groups (Table 3). The cPELD score was significantly higher in the TJP2-C group. Although the median age at liver biopsy among patients with the TJP2-C genotype (10.5 mo, IQR: 6–15.8) and TJP2-A/TJP2-B genotype (14 mo, IQR: 6.5–22.5) were comparable ($p = 0.409$), yet the histopathological findings were contrasting. Patients with TJP2-C (n = 10) were more likely to have portal/lobular inflammation with giant cell transformation (n = 7, 70%) and ductular proliferation with ductular bile plugs (n = 3, 30%). In contrast, patients with the TJP2-A and TJP2-B genotypes (n = 21) had a predominance of PILBD (9, 42.9%) and bland cholestasis (8, 38.1%). There was a trend towards a higher prevalence of advanced fibrosis (F3-F4 METAVIR) in patients with TJP2-C (7/10, 70%) than in the TJP2-A and TJP2-B genotypes (6/21, 28.3%) ($p = 0.052$). The outcome was significantly worse among patients with the TJP2-C genotype, with only 2 (12.5%) surviving with the native liver at the last recorded follow-up. While 9 (56.2%) patients received LT, 5 (31.3%) died on the waitlist. In contrast, NLS was significantly higher in patients with the TJP2-A (81%) and TJP2-B genotypes (71.4%). Among patients with TJP2-B, none died, and 2 (28.6%) received LT. Patients with TJP2-A (carrying missense mutations in both alleles) had the best outcome, with only 2 (9.5%) deaths and 2 (9.5%) patients needing LT. Log-rank analysis showed that NLS in patients with the TJP2-C genotype was significantly lower than those with the TJP2-A ($p < 0.001$) and TJP2-B ($p = 0.016$) genotypes (Figure 1). There was no difference in NLS between the TJP2-A and TJP2-B groups ($p = 0.583$) (Figure 1). Not only were patients with TJP2-C more likely to die or require LT, but they

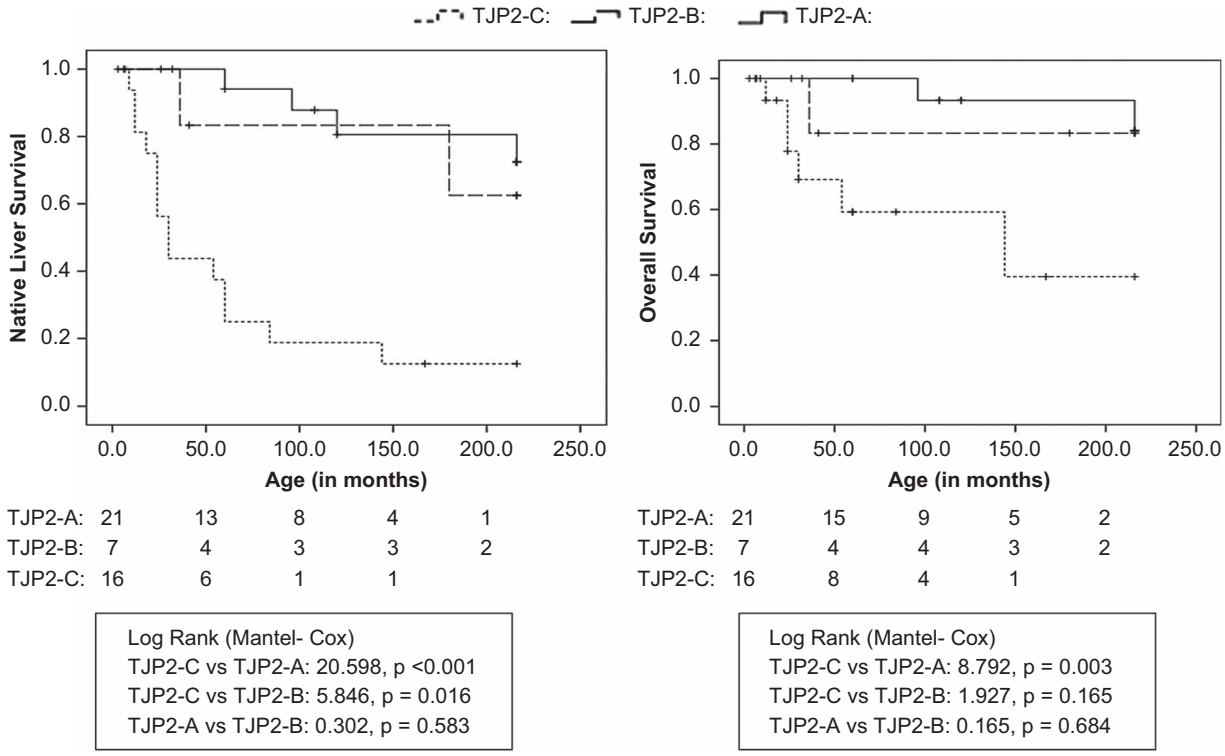

FIGURE 1 Kaplan-Meier survival curve depicting the native liver and overall survival based on the genotype category of patients with TJP2 deficiency. Native liver survival was significantly worse in patients with TJP2-C compared to patients with TJP2-A and TJP2-B. There was no difference in native liver survival between TJP2-A and TJP2-B. Overall survival was significantly lower in TJP2-C compared to TJP2-A genotype. Abbreviations: TJP2, tight junction protein 2; TJP2-A, tight junction protein 2 (missense mutations on both alleles); TJP2-B, tight junction protein 2 (missense mutation on one allele and a predicted protein-truncating mutation [PPTM] on the other); TJP2-C, tight junction protein 2 (PPTMs on both alleles).

did so at a much younger age (median: 2.5 y, IQR: 2–5) (Table 3). Of the 2 native liver survivors among the patients with TJP2-C, 1 child is only 18 months old at the time of last follow-up and is listed for LT due to progressive jaundice (latest total bilirubin: 17.5 mg/dL) and persistent pruritus (patient no. 15 in Table 2). The second child is 14 years old and clinically well, with the resolution of jaundice and pruritus after PIBD (patient no. 16 in Table 2). OS was significantly lower in patients with the TJP2-C genotype than in those with the TJP2-A genotype ($p = 0.003$). Although there was a trend toward lower OS in TJP2-C (62.5%) than in TJP2-B (85.7%), the difference was not statistically significant ($p = 0.165$).

We also performed a detailed analysis of the genotypes and outcomes of all patients with TJP2 deficiency reported to date in the literature (inclusion criteria: a minimum of 2 cases reported in the case series) (Table 4). The genotype correlated well with the outcome in the total cohort of 72 patients (TJP2-C: 40, TJP2-B+TJP2-A: 32), and TJP2-C was strongly associated with poor outcomes (OR for poor outcome (death/LT): 9 [95% CI: 3.08–26.33], $p < 0.001$). HCC has been exclusively reported in patients with the TJP2-C genotype.

SBD and its outcome

Thirty-four patients developed pruritus, of which 19 resolved with various combinations of antipruritic medications. Of the 15 patients (44.1%) who had refractory pruritus, 4 died while on the waitlist, and 6 underwent LT. Three patients underwent SBD at 3 different centers, and 2 patients with advanced fibrosis were listed for LT. In 2 of these patients, a genetic diagnosis was made after SBD. Two patients underwent PIBD (age at SBD: 24 and 36 mo) and 1 underwent partial external biliary diversion (age at SBD: 10 mo). The 2 patients undergoing PIBD showed complete resolution of pruritus, a significant decline in sBA (P1: 136 μmol/L → 8.7 μmol/L [6 mo after SBD]; P2: 158 μmol/L → 25.6 μmol/L [12 mo after SBD]), and are alive with their native liver at 6.5 and 11 years after SBD, respectively. One child who underwent partial external biliary diversion had only partial relief in pruritus, minimal decline in sBA (364 μmol/L → 186 μmol/L [minimum value after SBD]), and needed LT 1.2 years later for progressive jaundice and refractory pruritus.

TABLE 4 Genotype and associated outcome of patients with TJP2 deficiency–related cholestasis reported in literature till date[a]

Study, reference, total subjects	No. patients (TJP2-C)	Survival with native liver, n (%)	Death, n (%)	LT, n (%)	HCC, n (%)	No. patients (TJP2-A and TJP2-B)	Survival with native liver, n (%)	Death, n (%)	LT, n (%)	HCC, n (%)	OR (*p*)
	TJP2-C Genotype					TJP2-A and TJP2-B genotype					
Lal et al (present study), N = 44	16	2 (12.5)	5 (31.3)	9 (56.3)	0	TJP2-B: 7 TJP2-A: 21	5 (71.4) 15 (71.4)	— 2 (9.5)	2 (28.6) 4 (19)	0	
Sambrotta et al[6] N = 12	12	2 (16.7)	1 (8.3)	9 (75)		0					TJP2-C vs. other genotypes
Zhang et al[7] N = 7	4	4 (100)[b]			0	TJP2-B: 3	3 (100)			0	OR for poor outcome (death/LT): 9 (95% CI: 3.08–26.33), $p < 0.001$
Menon et al[25] N = 4	4			4 (100)	1 (25)	0					
Tang et al[8] N = 3	2	2 (100)			1	TJP2-B: 1	1 (100)			0	OR for the development of HCC: cannot be calculated as no event in the TJP2-A and TJP2-B groups, $p = 0.124$
Zhou et al[15] N = 2	2	0		2 (100)	2 (100)	0					
Total N = 72	40	10 (25)	6 (15)	24 (60)	4 (10)	32	24 (75)	2 (6.3)	6 (18.8)	0	

Note: Categorization of TJP2 genotype: TJP2-A: carrying missense mutations on both the alleles; TJP2-B: carrying missense mutation on 1 allele and PPTMs on the other; TJP2-C: carrying PPTMs on both the alleles.
[a]Including the case series describing a minimum of 2 cases.
[b]Outcomes described only till 4 years of age in 6 out of 7 patients.
Abbreviations: LT, liver transplantation; PPTM, predicted protein-truncating mutations; TJP, tight junction protein.

LT and its outcomes

Thirteen (29.5%) patients underwent LT at a median age of 36 months (IQR: 24–60). The majority of the patients who underwent LT were TJP2-C (9/13, 69.2%). The most common indications for LT were decompensated cirrhosis (n = 7; 53.8%), progressive jaundice, refractory pruritus (n = 4; 30.7%), and intractable pruritus with growth failure (n = 2; 15.4%). One patient underwent deceased donor LT, whereas 12 (92.3%) received organs from a relative (living donor LT). At a median follow-up of 12 months (IQR: 8–24), 11 (84.6%) patients were alive with a healthy graft, while 2 (15.4%) died in the perioperative period. Major post-LT complications included acute rejection in 5 (38.5%), recurrent diarrhea in 1 (7.7%), and neurological injury in 1 (7.7%). Catch-up growth was observed in 9 of the 11 (81.8%) surviving patients at the last follow-up. None of the explants had HCC.

Predictors of outcome

We aimed to evaluate whether any parameter at the initial presentation or early in the clinical course could predict the outcome. Children surviving with their native liver but followed up for <18 months (n = 4) were excluded from this analysis as their outcomes may change with a longer follow-up. Thus, 20 patients who died or underwent LT were included in the poor-outcome group, whereas 20 patients with NLS were included in the good-outcome group. In univariate analysis, patients with poor outcomes were more likely to have the TJP2-C genotype ($p < 0.001$), failed to show resolution of jaundice during medical therapy ($p < 0.001$), and decompensated with ascites ($p < 0.001$). Patients with poor outcomes also had higher bilirubin levels ($p = 0.003$), INR ($p = 0.009$), cPELD ($p = 0.001$), and sBA ($p = 0.004$) and lower albumin levels ($p = 0.006$) (Table 5). As there were only 20 events (death/LT) in the cohort, limiting the number of variables that could be entered simultaneously in the Cox regression analysis, we used cPELD, which includes bilirubin, INR, and albumin levels, instead of individual biochemical parameters. Two models were evaluated using Cox regression: the first included 4 variables (genotype categorization, ascites, resolution of jaundice, and cPELD), and the second included genotype categorization, ascites, resolution of jaundice, and sBA. Cox regression analysis using both models identified the TJP2-C genotype (2 PPTMs) (HR: 14.1, 95% CI: 2.4–81.8, $p = 0.003$) and resolution of jaundice (HR: 0.12, 95% CI: 0.02–0.99, $p = 0.049$) as independent predictors of outcome.

DISCUSSION

This is the largest reported registry database of patients with TJP2 deficiency. Most patients with TJP2 present with cholestasis during early infancy. Pruritus is common and often refractory to medications that require SBD. The finding of PILBD in approximately one-fourth of liver histopathology specimens was a novel finding. Most importantly, the study demonstrated a strong genotype-phenotype correlation that could reliably predict the clinical course and outcome of a patient at the time of presentation. Patients with the TJP2-C genotype (carrying PPTMs on both alleles) presented early and almost universally decompensated and died if they failed to receive timely LT. Patients with TJP2-C decompensated early (median, 18 mo; IQR: 6.5–24) and died or received LT at a very young age (median age at LT/death: 2.5 y, IQR: 2–5 y). The post-LT outcomes were good.

TJP2 deficiency was the third most common cause of genetic intrahepatic cholestasis among the patients in the registry. With the advent of genetic diagnosis and its widespread accessibility, TJP2 deficiency is being increasingly diagnosed and accounts for as many as 12%–23% of children presenting with the phenotypic spectrum of PFIC.[4,5,22,23] Jaundice was the earliest presenting symptom at a median age of 2 months (range: 1–6 mo), followed by pruritus at a median age of 8 months (IQR: 4.3–12 mo). All 12 patients reported by Sambrotta et al and 6 out of 7 (85.7%) reported by Zhang et al presented with cholestasis within the first 3 months of life.[6,7] The early age of onset of jaundice and pruritus coupled with rapidly progressive fibrosis and progression to end-stage liver disease makes this condition almost indistinguishable from bile salt exporter pump deficiency.[24] The present study definitively established a distinct genotype-phenotype correlation. Patients with genotypes categorized as TJP2-C (biallelic PPTMs) showed a rapidly progressive disease course and poor outcomes. Fourteen of the 16 (87.5%) patients with TJP2-C died or underwent LT at a very young age (median: 2.5 y). Zhang et al[7] demonstrated that patients with biallelic PPTMs had complete loss of function of the TJP2 protein, whereas patients with PPTM on 1 allele and missense variant on the other retained partial TJP2 expression, possibly explaining the severe disease observed in patients with biallelic PPTMs. In contrast, a milder phenotype was observed in patients categorized as TJP2-A and TJP2-B, characterized by high rates of resolution of jaundice (52.2%) and pruritus (71.4%) on medical therapy. Genotype categorization was an independent and early predictor of poor outcomes on Cox regression analysis (HR for TJP2-C: 14.1, 95% CI: 2.4–81.8). The correlation between genotype and outcome is also evident from the combined analysis of the cases reported in the present study and those reported in the literature, as shown in Table 4.[6–8] Only 2 of 12 patients (16.6%) with biallelic PPTMs reported by Sambrotta et al[6] survived with their native liver at their last reported follow-up. On the other hand, Zhang and colleagues described a mixed cohort comprising 4 patients with 2 PPTMs and 3 patients with 1 PPTM and found that patients with 2

TABLE 5 Univariate and Cox multivariate regression analysis to identify the predictors of outcome in patients with TJP2 deficiency (good outcome: survival with native liver; poor outcome: death or liver transplantation)

Parameter	Poor outcome (n = 20)	Good outcome (n = 20)[a]	Significance (nonparametric) *p*	Cox regression analysis (adjusted HR, 95% CI), *p*
Genotype categorization, n (%)				
TJP2-A	4 (20)	14 (70)		*HR for poor outcome: TJP2-C vs. others 14.1 (2.4–81.8) p = 0.003*
TJP2-B	2 (10)	4 (20)	< 0.001	
TJP2-C	14 (70)	2 (10)		
Gender, n (%)				
Male	12 (60)	11 (55)	1.000	
Jaundice, n (%)	19 (95)	16 (80)	0.342	Not included in Cox regression
Age at the onset of jaundice (mo)	2.5 (1–6.3)	3 (1.3–5.3)	0.756	
Resolution of jaundice, n (%)	1/19 (5.3%)	12/16 (75%)	< 0.001	*HR for poor outcome: 0.12 (0.02–0.99) p = 0.049*
Ascites, n (%)	15 (75)	0	< 0.001	*Variable not in the equation*
Age at the onset of ascites (mo)	24 (10.5–78)	NA	NA	
Pruritus, n (%)	17 (85)	16 (80)	1.000	
Age of onset in pruritus (mo)	10 (7–24)	6.5 (4–12)	0.196	
Refractory pruritus, n (%)	11/17 (64.7)	3/16 (18.7)	0.09	
Growth failure, n (%)	13 (65)	11 (55)	0.748	
Hepatopulmonary syndrome, n (%)	4 (20)	0	0.13	Not included in Cox regression
Bilirubin (mg/dL)	13.2 (5–17.5)	2.1 (0.7–8.2)	0.003	
INR	1.3 (1.1–1.6)	1.02 (1–1.3)	0.009	
Alanine aminotransferase (IU/L)	92 (50.5–286)	64 (43–102)	0.159	
Albumin (g/dL)	3.6 (2.8–3.9)	4 (3.7–4.3)	0.006	
Alfa-fetoprotein (ng/mL)	54 (8.1–211.5)	649.9 (1.8–7463)	0.424	
Significant fibrosis (F3-F4 METAVIR), n (%)	7/10 (70)	6/21 (28.6)	0.052	
Serum bile acids (μmol/L)	231.5 (179.3–316)	90 (25–213)	0.004	*Variable not in the equation*
Calculated PELD score	17.8 (12.9–24.4)	6 (2–12.3)	0.001	*Variable not in the equation*

Note: Categorization of TJP2 genotype: TJP2-A: carrying missense mutations on both the alleles; TJP2-B: carrying missense mutation on 1 allele and PPTM on the other; TJP2-C: carrying PPTMs on both the alleles.
Iitalic values indicates are statistically significant.
[a]Children surviving with their native liver but followed up till less than 18 months of age (n = 4) were excluded from this analysis as their outcome may change with longer follow-up.
Continuous variables are expressed as median (IQR) and categorical values as proportions. For univariate analysis, the Mann-Whitney *U* test was used to compare the continuous variables, and the Fisher exact test to compare the categorical variables. Cox multivariate regression analysis was performed to identify the independent predictors of outcome.
Abbreviations: NA, not applicable; PELD, pediatric end-stage liver disease score; PPTM, predicted protein-truncating mutations; TJP2, tight junction protein 2.

PPTMs had a higher prevalence of growth failure, although surprisingly, all their patients, irrespective of the genotype, had mild cholestasis and all survived with their native liver until the last follow-up. It should be noted that none of the patients in the cohort by Zhang et al[7] were followed up beyond 4 years of age to assess the long-term outcomes. At present, there is a limited understanding of the reasons for this difference in outcomes across studies, and the role of ethnicity cannot be ruled out. Indirect evidence of a progressive clinical course in patients with TJP2-C can also be derived from the fact that all patients undergoing LT had 2 PPTMs in the cohort of patients with TJP2 reported by Menon et al.[25] Carlton et al[26] reported mild cholestasis with a benign course accompanied by elevated sBA in Amish patients with missense variants in both *TJP2* and *bile acid Coenzyme A: amino acid N-acyltransferase*, which was labeled as familial hypercholanemia. Patients with missense mutations may remain asymptomatic during childhood and present with

cholestasis for the first time in adulthood.[11] Intrahepatic cholestasis of pregnancy has been described in women with heterozygous variants and even in a few women with missense homozygous variants in TJP2.[9,10]

TJP2 deficiency may theoretically present with several extrahepatic manifestations, as tight junctions are ubiquitous in every epithelial cell.[3] The various extrahepatic manifestations reported include sensorineural deafness, neurological and respiratory disorders, renal tubular damage, and hypomyelination, and myopia, which often fail to revert after LT.[6,12–14] There was no association between extrahepatic symptoms and the genotype in the present study. The extrahepatic manifestations reported in the literature are not severe enough to be considered a contraindication for LT.[6,12–14] Liver histopathology findings in TJP2 are variable and usually demonstrate predominant portal/lobular inflammation and cholestasis.[6,7] Immunohistochemical studies on liver tissue specimens usually demonstrate an absence of TJP2 and markedly reduced as well as improperly localized claudin 1.[6,7] None of the participating centers in this registry currently perform TJP2 or claudin 1 immunostaining. A peculiar finding in this study was the presence of PILBD in approximately one-fourth of the liver histology specimens. Pruritus was common (76.7%) and often refractory (45.5%). Complete resolution of pruritus was observed in 2 of 3 children undergoing SBD. Genotype does not seem to play a role in predicting post-SBD outcomes; however, the number of patients undergoing SBD is too small to make any definitive interpretation. A significant reduction in sBA following SBD was observed in responders, similar to the trend reported in patients with bile salt exporter pump.[24] The ileal bile acid transporter inhibitor odevixibat, which is now available in many parts of the world, acts through a mechanism similar to that of SBD by interrupting the enterohepatic circulation of bile acids, thus lowering sBA.[27] Odevixibat has been used in patients with TJP2 deficiency, with a complete response in pruritus and a significant decrease in sBA.[27] Ileal bile acid transporter inhibitors are likely to be preferred for patients with pruritus, while SBD may be reserved for those parts of the world where this drug is not available.

This study included the largest cohort of patients who underwent LT for TJP2 deficiency. Graft and patient survival rates were favorable (84.6%) at a median follow-up of 12 months, with catch-up growth and no recurrence of disease. Favorable post-LT outcomes have also been reported in published case series.[25] Most of the patients requiring LT from the registry database, as well as those reported in the literature, are those who would be categorized as the TJP2-C genotype.[5,24] The early development of HCC is a peculiar feature of TJP2, exclusively reported in patients with PPTMs (Table 4).[12,15,16] However, none of our patients were diagnosed with HCC, including the evaluation of 12 explant specimens. It is worth emphasizing that these tumors may remain asymptomatic and may only be detected by imaging or histological evaluation of explants.[12,15,16] Thus, routine follow-up of patients with alfa-fetoprotein and ultrasound screening for liver lesions is essential.

The strength of this study is that it is the largest and only multicenter study describing the clinical course and outcomes of TJP2 disease in detail. The study was limited by the retrospective nature of data collection. Another limitation was that the parents of some probands with compound heterozygous mutations did not undergo genetic analysis. We also need a longer duration of follow-up after LT to understand long-term graft survival, recognize any unique complications, and understand the impact of extrahepatic manifestations on quality of life after LT. Based on the findings of this study, we concluded that TJP2 deficiency presents as cholestasis at a young age. The genotype of a patient can reliably predict its clinical course and outcomes. Patients with the TJP2-C genotype carrying PPTMs on both alleles have a rapidly progressive course leading to decompensation in early childhood and die within the first 5 years of life if they do not receive timely LT. Outcomes after LT are favorable. The response to medical therapy was favorable in patients with TJP2-A or TJP2-B genotypes.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author and can be obtained upon request.

AUTHOR CONTRIBUTIONS

Bikrant Bihari Lal: Conceptualization of the study, design of the study, data collection, analysis and interpretation of data, writing the first draft, revising the manuscript, and approval of the final version. Seema Alam, Nirmala Dheivamani, Aabha Nagral, Nishant Wadhwa, Yogesh Waikar, Vikrant Sood, and Rajeev Khanna: Conceptualization of the study, design of the study, data collection, revision of the manuscript with important intellectual content, and approval of the final version. Anupam Sibal, Karunesh Kumar, HR Somashekara, Ashish Bavdekar, Arjun Maria, Aashay Shah, Ira Shah, Zahabiya Nalwalla, K.P. Srikanth, Subhash Gupta, Viswanathan M. Sivaramakrishnan, and Arya Suchismita: Study design, data collection, manuscript revision with important intellectual content, and approval of the final version. Vaibhav Shah: Study design, data collection, critical revision of the manuscript with important intellectual content, and approval of the final version. Snehavardhan Pandey: Conceptualization of the study, design of the study, data collection and analysis, revision of the manuscript with important intellectual content, and approval of the final version. A. Ashritha: Conceptualization of the study, design of the study, data collection, analysis and interpretation of data, revision of the manuscript with important intellectual content, and approval of the final version.

CONFLICTS OF INTEREST

The authors have no conflicts to report.

ETHICS APPROVAL

The study was approved by the Institutional Ethics Committee of the Institute of Liver and Biliary Sciences, New Delhi (IEC/2022/93/MA14). The study has been registered with clinicaltrials.gov (NCT05704517).

PATIENT CONSENT STATEMENT

A consent waiver was obtained as this was a retrospective study.

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