

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2020 February 7; 26(5): 456-561



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The WJG is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2019 edition of Journal Citation Report® cites the 2018 impact factor for WJG as 3.411 (5-year impact factor: 3.579), ranking WJG as 35<sup>th</sup> among 84 journals in gastroenterology and hepatology (quartile in category Q2). CiteScore (2018): 3.43.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Responsible Electronic Editor: *Yu-Jie Ma*  
 Proofing Production Department Director: *Yun-Xiaoqian Wu*

**NAME OF JOURNAL**

*World Journal of Gastroenterology*

**ISSN**

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

**LAUNCH DATE**

October 1, 1995

**FREQUENCY**

Weekly

**EDITORS-IN-CHIEF**

Subrata Ghosh, Andrzej S Tarnawski

**EDITORIAL BOARD MEMBERS**

<http://www.wjgnet.com/1007-9327/editorialboard.htm>

**EDITORIAL OFFICE**

Ze-Mao Gong, Director

**PUBLICATION DATE**

February 7, 2020

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**PUBLICATION MISCONDUCT**

<https://www.wjgnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## New tight junction protein 2 variant causing progressive familial intrahepatic cholestasis type 4 in adults: A case report

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**Author contributions:** Wei CS, Vogel I, and Grønbaek H designed the study and collected data; Becher N, Vogel I and Blechingberg J performed genetic testing and consultation; Ott P supervised manuscript writing and the histological evaluations; Wei CS, Becher N and Grønbaek H analyzed the patients' data; Wei CS wrote the manuscript; all authors critically revised the manuscript; all authors reviewed the manuscript and approved the final version.

**Supported by** Sanming Project of Medicine in Shenzhen of China, No. SZSM201612074.

**Informed consent statement:** Written informed consent was obtained from the index patient and all individuals of the family in Denmark for the genetic study and publication of this case report.

**Conflict-of-interest statement:** All other authors declare that they have no conflicts of interest.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**Open-Access:** This article is an

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### Abstract

#### BACKGROUND

Progressive familial intrahepatic cholestasis (PFIC) encompasses a group of autosomal recessive disorders with high morbidity and mortality. Variants in the gene encoding tight junction protein-2 (TJP2) have been linked to PFIC type 4 (PFIC4), which predominantly presents in childhood. However, there are only limited data from adults with TJP2-related PFIC4. We report a family with an autosomal recessive disorder with a novel variant in the *TJP2* gene in adults with very variable expression of PFIC4.

#### CASE SUMMARY

The index patient presented at 19 years old with liver cirrhosis and variceal bleeding and was treated with endoscopic banding and beta-blockers. In 2018, he developed primary liver cancer that was treated with radiofrequency ablation followed by liver transplantation in 2019. Genetic testing revealed a novel homozygous TJP2 variant causing PFIC4 (TJP2([NM\_004817.3]:c.[3334C>T]; [3334C>T])). The consanguineous family consists of the father and mother (both heterozygous) and their 12 children, of which five carry the variant in a homozygous state; however, these five siblings have highly variable expression of PFIC4. Two homozygous brothers had cirrhosis and portal hypertension at diagnosis at the ages of 19 and 36. Two other homozygous brothers, age 23 and 19, and the homozygous sister, age 21, have elevated liver enzymes but presently no cirrhosis, which may suggest an age-dependent penetrance. In addition, five sisters had severe and mild intrahepatic cholestasis of pregnancy and carry the TJP2 variant in a homozygous and heterozygous state, respectively.

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**Manuscript source:** Invited manuscript

**Received:** November 18, 2019

**Peer-review started:** November 18, 2019

**First decision:** December 23, 2019

**Revised:** January 12, 2020

**Accepted:** January 19, 2020

**Article in press:** January 19, 2020

**Published online:** February 7, 2020

**P-Reviewer:** Ali FEM, Jin C, Mizuguchi T, Norton PA, Zhu YY

**S-Editor:** Ma YJ

**L-Editor:** A

**E-Editor:** Ma YJ



## CONCLUSION

This novel TJP2 variant is associated with PFIC4 causing severe liver disease with cirrhosis and primary liver cancer in adolescents/adults.

**Key words:** Progressive familial intrahepatic cholestasis; Tight junction protein 2; Genetic variants; Liver cirrhosis; Liver cancer; Case report

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**Core tip:** We report a family with progressive familial intrahepatic cholestasis type-4 (PFIC4) with a novel variant in the tight junction protein-2 gene. The five homozygous children have highly variable expression of PFIC4. Two brothers presented with cirrhosis, portal hypertension and hepatocellular carcinoma at ages 19 and 36. Two other homozygous brothers and a sister (age 19-23) have elevated liver enzymes without cirrhosis. Furthermore, five sisters had severe and mild intrahepatic cholestasis of pregnancy and carry the tight junction protein-2 variant in homozygous and heterozygous states, respectively. Close monitoring for cirrhosis and hepatocellular carcinoma development is warranted in PFIC4 syndrome.

**Citation:** Wei CS, Becher N, Blechingberg J, Ott P, Vogel I, Grønbaek H. New tight junction protein 2 variant causing progressive familial intrahepatic cholestasis type 4 in adults: A case report. *World J Gastroenterol* 2020; 26(5): 550-561

**URL:** <https://www.wjgnet.com/1007-9327/full/v26/i5/550.htm>

**DOI:** <https://dx.doi.org/10.3748/wjg.v26.i5.550>

## INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) encompasses a group of autosomal recessive disorders with high mortality and morbidity in childhood. There are few reports of the exact incidence of PFIC, but it is estimated to be 1/50000 to 1/100000<sup>[1]</sup>. Variants in the ATP-transporter genes ATP8B1, ABCB11, and ABCB4 are responsible for PFIC types 1, 2 and 3, respectively, and recently, the gene encoding tight junction protein-2 (TJP2) has been linked to PFIC type 4 (PFIC4), which predominantly presents in childhood<sup>[2]</sup>. However, there are only limited data from adults with TJP2-related PFIC4.

The TJP2 gene was first discovered by Gumbiner *et al*<sup>[3]</sup> in 1991. The TJP2 gene is located on chromosome 9q21.11 and has a total of 80932 base pairs and includes 23 exons<sup>[4]</sup>. The TJP2 gene product is the tight junction protein 2, also called zona occludens 2 (ZO-2), which belongs to the membrane-associated guanylate cyclase family<sup>[5]</sup>. TJP2 stabilizes tight junctions by binding to the cytoplasmic C termini of junctional transmembrane proteins, such as claudins, and linking them to the actin cytoskeleton<sup>[6]</sup>. The hepatocyte membranes adjacent to each other at the junction of the bile duct canaliculi and the hepatocyte basal membrane surface form a tight junction essential for the separation of bile from plasma<sup>[7,8]</sup>. Loss-of-function variants in the TJP2 gene can cause defects in tight junction function, leading to severe cholestatic liver disease, along with extrahepatic manifestations<sup>[9]</sup>.

Here, we report a family with a novel TJP2 variant. The index patient, a 20-year-old male, was diagnosed with cirrhosis and portal hypertension in 2015 due to unexplained intrahepatic cholestasis. Based on the family history, we performed genetic screening of the index patient, eight of his eleven siblings and his parents. We discovered a novel TJP2 gene variant (TJP2 [NM\_004817.3]:c.[3334C>T];[3334C>T] (p.(Gln1112\*)) causing liver disease when both alleles were affected. Here, we report the clinical history.

## CASE PRESENTATION

### *Clinical and biochemical data of the index patient and family*

**Basic information:** The family consists of the parents (P1.1-P1.2), 12 live siblings (P2.1-P2.12) and two siblings who died at birth (P2.13-2.14); and 20 grandchildren (P3.1-P3. 20) as depicted in [Figure 1](#).



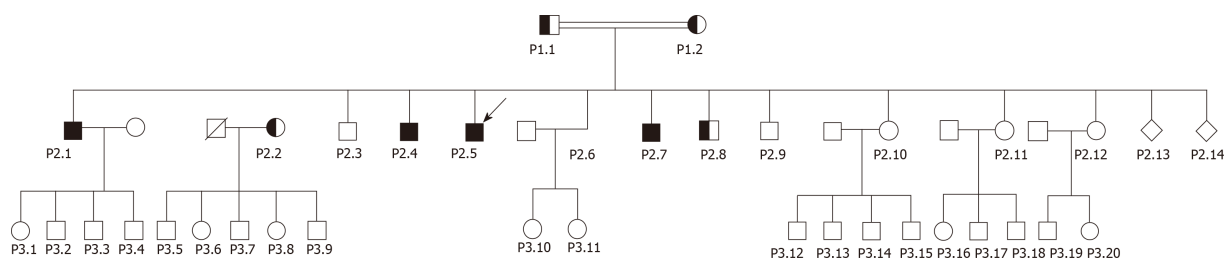


Figure 1 The family pedigree of the tight junction protein 2 variant.

### Case 1: Index patient (P2.5)

**Chief complaints:** In 2015, the index patient (P2.5), a 20-year-old male, presented at our department with decompensated liver cirrhosis with previous variceal bleeding and complaints of tiredness.

**History of present illness:** At age 19, he had variceal bleeding treated with endoscopic banding and beta-blocker as secondary prophylaxis. In 2018 he developed biopsy-proven hepatocellular carcinoma (HCC) treated with radiofrequency ablation, and in 2019, he had a liver transplantation.

**Physical examination:** At first visit the patient was in good performance status and had normal BMI. He had clinical signs of splenomegaly and mild icterus but otherwise a normal physical examination with no signs of ascites.

**Laboratory examinations:** He presented with elevated bilirubin, alkaline phosphatase, decreased serum albumin (ALB) and normal alanine aminotransferase (ALT) as shown in Table 1.

In 2015, a liver biopsy showed established cirrhosis that was etiologically nonspecific but may include cirrhosis due to a cholestatic liver disease. In the fibrous septae, there were findings of original bile ducts and some bile duct proliferation accompanied by light to moderate etiologically nonspecific lymphocytic inflammation with only a few plasma cells. There were no granulomas and only very mild focal interphase hepatitis. A liver biopsy in 2018 confirmed the HCC diagnosis found on CT scanning.

**Imaging examinations:** A CT scan at first visit showed liver cirrhosis, splenomegaly, and portosystemic collateral blood flow. A CT scan in 2018 showed a 3 cm HCC, which was treated with RFA and with no signs of recurrence on follow-up CT scans.

Liver vein catheterization showed a hepatic venous pressure gradient of 12 mmHg on beta-blocker treatment consistent with significant portal hypertension.

**History of past illness:** He had been told of transient icterus a few months after birth after an uncomplicated pregnancy. The remaining childhood was uneventful with normal development and milestones.

## FINAL DIAGNOSIS

The patient was diagnosed with the PFIC4 syndrome of liver cirrhosis and portal hypertension with variceal bleeding and later HCC caused by a mutation in the *TJP2* gene.

## TREATMENT

The portal hypertension and varices were treated with endoscopic band ligation and beta-blocker treatment. The HCC was initially treated with radiofrequency ablation and finally the patient received a successful liver transplantation.

## OUTCOME AND FOLLOW-UP

The patient is in good performance status and health, and in follow-up after successful liver transplantation.

**Table 1 Clinical and biochemical parameters and FibroScan data of tested family members on the day of the first presentation**

TJP2 status	P1.1	P1.2	P2.1	P2.2	P2.3	P2.4	P2.5	P2.6	P2.7	P2.8	P2.9	P2.10-12
	+/wt	+/wt	+/+	+/wt	wt/wt	+/+	+/+	+/+	+/+	+/wt	+/+	NA
Gender	M	F	M	F	M	M	M	F	M	M	M	F, F, F
Age in 2018 (yr)	60	55	36	32	31	25	23	21	19	16	15	NA
ALAT (< 45 female; < 70 male; (U/L)	16	19	114	15	10	43	37	44	97	21	22	NA
Bilirubin (5-25 µmol/L)	8	4.7	29	4.4	14	12	45	10	17	14	11	NA
Alkaline phosphatase (35-105 U/L)	101	104	120	65	50	112	212	117	115	104	121	NA
GGT (10-80 U/L)	72	21	61	14	12	44	156	55	86	12	15	NA
Coagulation II, VII, X/INR	1.0	1.2	1.2	0.81	1	1.2	1.7	1.1	0.73	0.75	0.72	NA
Albumin (36-48 g/L)	44	41	36	37	44	46	29	37	27	44	47	NA
Creatinine (60-105 µmol/L)	68	40	71	49	62	68	66	37	71	67	53	NA
Cholesterol (< 5.0 mmol/L)	5.7	6.5	5	4.3	3.4	3.8	3.2	4.4	NA	3.3	4.7	NA
HDL cholesterol (> 1.0 mmol/L)	0.91	1.1	1.8	2.0	0.92	2	1.7	1.9	NA	1.1	1.3	NA
LDL cholesterol (< 3 mmol/L)	3.3	3.7	2.7	1.9	2.2	1.4	1.1	2.2	NA	1.8	3	NA
Triglyceride (< 2 mmol/L)	3.3	3.5	1.1	0.9	1.1	1	1	0.7	NA	0.9	1	NA
Leucocytes (3.5-10.0 10 <sup>9</sup> /L)	11.7	4.72	4.19	6.96	5.8	7.03	4.25	6.44	5.78	7.8	5.7	NA
Hemoglobin (> 7.3 female; > 8.3 male, mmol/L)	9.4	7.8	9.3	7.5	9.9	10.1	7.8	8	9.9	10.3	9.6	NA
Platelets (165-400, × 10 <sup>9</sup> /L)	335	405	77	266	229	218	64	191	210	379	339	NA
sCD163 (0.69-3.86 mg/L)	2.32	1.94	NA	1.90	2.95	3.96	5.23	6.03	3.91	1.31	1.75	NA
FibroScan (kPa)	4.4	3.3	75	4-7	6.6	8.1	24	9.1	5.9	5.7	7.8	NA

TJP2 status is indicated by +/+ (homozygous) +/WT (heterozygous), and wt/wt indicating wild type. NA: Not applicable; WT: Wild type.

### Family history

This Syrian family immigrated to Denmark in 2015. Both parents and nine of their 12 children live in Denmark, while three children live in the Middle East. The three siblings living outside Denmark are apparently healthy with no liver-related symptoms, but all three have a history of mild pruritus during pregnancy (G4+G3+G2), suggesting intrahepatic cholestasis of pregnancy (ICP). However, the information is limited, and no genetic, clinical or biochemical information is available. All 20 children of the third generation (all below the age of 18) have no apparent liver-related symptoms.

#### Case 2-3: Parents (P1.1-P1.2): Consanguineous, unspecified.

P1.1: The father, 60 years old in 2018, and heterozygous for the TJP2 variant, has no liver-related complaints and normal liver biochemistry. He has slightly elevated cholesterol and triglyceride levels.

P1.2: The mother, 55 years old in 2018, and heterozygous for the TJP2 variant, has no liver-related symptoms and normal liver biochemistry. She has slightly elevated cholesterol and triglyceride levels. No history of intrahepatic cholestasis or pruritus during 14 pregnancies. Twelve children are alive, and two died in the perinatal period (P2.13-2.14) without any information on liver disease.

**Case 4-11: Siblings to the index case.** The index case (P2.5) has 11 living siblings (P2.1-P2.4 and P2.6-P2.12), and two siblings (P2.13-P2.14) who died at birth. Three of the 11 live siblings (P2.10-P2.12) do not live in Denmark and could not be studied. The remaining siblings were studied.

P2.1: Male, 36-year-old, homozygous for the TJP2 variant, presented in 2018 with abdominal pain and pruritus, elevated liver parameters, bile acids and pruritus (Table 1). Ultrasound showed splenomegaly. Due to clinical decompensation with ascites and worsening liver function with increasing ALT 224 U/L, alkaline phosphatase 191 U/L, bilirubin 434 µmol/L and INR 2.6, he was diagnosed with severe acute-on-chronic liver failure (ACLF) and received successful liver transplantation. The patient is married and has 4 children, all apparently healthy but heterozygous for the variant.

Biopsy of the explanted liver showed established cirrhosis with regeneration noduli with bile duct proliferation and inflammation in the fibrous septae. There was pronounced canalicular cholestasis. There were no granulomas.

P2.2: Female, 32-year-old in 2018, heterozygous for the TJP2 variant. She has no liver-related symptoms and normal liver biochemistry. During all five uncomplicated

pregnancies, she suffered from mild pruritus with no evidence of elevated liver enzymes and received no treatment with ursodeoxycholic acid. She has five healthy children.

P2.3: Male, 31 years old in 2018, carries two wild-type TJP2 alleles and has no liver-related symptoms and normal liver biochemistry.

P2.4: Male, 25-year-old in 2018, homozygous for the TJP2-variant. He had neonatal icterus, but apart from that otherwise healthy. He has slightly elevated alkaline phosphatase and normal ALT and bilirubin.

Liver biopsy 2018 showed normal liver histology with no signs of cholestatic liver disease.

P2.6: Female, 18-year-old in 2016, homozygous for the TJP2-variant. In 2016, she was asymptomatic but had elevated liver alkaline phosphatase (117 U/L). At age 21, she was pregnant, and in pregnancy week 10, she had slightly elevated alkaline phosphatase. From pregnancy week 23, she developed pruritus and increased ALT (91 U/L), alkaline phosphatase (235 U/L), and bile acid levels (57 mmol/L) but normal bilirubin. She was treated with ursodeoxycholic acid from pregnancy week 29 (250 mg t.i.d. increased to 500 mg b.i.d.) with effect on pruritus and bile acids. After spontaneous delivery at gestational week 37+4 of a healthy girl, the liver parameters normalized.

In August 2018, a new pregnancy was complicated with pruritus and elevated alkaline phosphatase (129 U/L) and bile acids (35  $\mu$ mol/L) from week 17 but normal ALT and bilirubin levels. She was treated with ursodeoxycholic acid (500 mg t.i.d.) and rimactan (300 mg/day) for severe pruritus until labor was induced at week 38, and she delivered a healthy daughter with no signs of cholestatic liver disease.

A liver biopsy in 2017 between two pregnancies showed minimal unspecific changes with minor lymphocytic infiltration and no ductopenia. She has elevated sCD163 (6.03 mg/L) and a FibroScan of 9.1 kPa.

P2.7: Male, 19-year-old in 2018, homozygous for the TJP2 variant, has no symptoms of liver disease. He has slightly elevated liver enzymes and a FibroScan of 5.9 kPa and sCD163 3.91 mg/L.

P2.8: Male, 16-year-old in 2018, heterozygous for the TJP2 variant, has no symptoms of liver disease and normal liver enzymes and FibroScan.

P2.9: Male, 15-year-old male in 2018, carries two wild-type TJP2 alleles, has no symptoms of liver disease and normal liver enzymes and a FibroScan of 7.8 kPa.

**Case 12-34:** P2.10-P2.12: Living outside Denmark and are apparently without symptoms of liver disease; however, all three women with 4, 3 and 2 pregnancies have reported pruritus in their pregnancies. Blood samples and genetic testing have not been performed.

P3.1-P3.20: Apparently without symptoms of liver disease but limited information available.

### Other gene variants

As we used a gene panel approach, in addition to the TJP2 variants, we also discovered a number of other variants in genes potentially involved in genetic cholestatic liver diseases, ABCB11, ABCG5, ABCC2 and UGT1A1, as shown in [Table 2](#).

### Genetic testing

DNA from the index patient and his family members was screened for variants in genes known to be involved in a spectrum of liver and cystic diseases using an in-house-targeted gene panel. The following genes were analyzed: *ATP8B1*, *ABCB11*, *ABCB4*, *ABCG5*, *ABCC2*, *JAG1*, *NOTCH2*, *UGT1A1* and *TJP2*.

Genomic DNA was isolated from peripheral blood leukocytes using the magnetic bead-based automated Chemagic MSM1 instrument, following the manufacturer's instructions (PerkinElmer Chemagen, Baesweiler, Germany). A library for Illumina paired-end sequencing was constructed from 1  $\mu$ g of genomic DNA using the KAPA HTP Library Preparation Kit according to the manufacturer's instructions (KAPA Biosystems Inc., Wilmington, MA). The generated libraries were enriched for regions of interest using a customized targeting probe set (SeqCap EZ Choise, Roche Nimblegen, Inc., Madison, WI) and sequenced on a MiSeq Desktop Sequencer (Illumina, San Diego, CA). The reads obtained from sequencing were aligned to the human genome (hg19), and variants were called using Biomedical Genomics Workbench v.2 (CLC Bio-Qiagen, Aarhus, Denmark).



Table 2 Genetic variations discovered in the family using the gene panel approach

Patient ID	Age (yr)	Gender	TJP2 variation	TJP2 genotype	Other gene variations	Phenotype
P2.1	36	M	TJP2 [NM_004817.3]:c.[3334C>T];[3334C>T]	Homozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[=] <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=] <i>UGT1A1</i> [NM_000463.2]C.-53TA[6];[7]	PFIC4 with cirrhosis, portal hypertension and acute-on-chronic liver failure; Liver transplantation
P2.5	23	M	TJP2 [NM_004817.3]:c.[3334C>T];[3334C>T]	Homozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[1331T>C] <sup>2</sup> <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=]	PFIC4 with cirrhosis, portal hypertension. Primary liver cancer; Liver transplantation
P2.4	25	M	TJP2 [NM_004817.3]:c.[3334C>T];[3334C>T]	Homozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[1331T>C] <sup>2</sup> <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=]	Elevated liver enzymes
P2.6	21	F	TJP2 [NM_004817.3]:c.[3334C>T];[3334C>T]	Homozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[=] <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=] <i>UGT1A1</i> [NM_000463.2]C.-53TA[6];[7]	Elevated liver enzymes; Severe cholestasis in pregnancy
P2.7	19	M	TJP2 [NM_004817.3]:c.[3334C>T];[3334C>T]	Homozyg-ous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[=] <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=] <i>UGT1A1</i> [NM_000463.2]C.-53TA[6];[7]	Elevated liver enzymes
P1.1	60	M	TJP2 [NM_004817.3]:c.[3334C>T];[3334=]	Heterozyg-ous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[1331T=] <i>ABCC2</i> [NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup> <i>UGT1A1</i> [NM_000643.2]C.-53TA[6];[7]	Normal
P1.2	55	F	TJP2 [NM_004817.3]:c.[3334C>T];[3334=]	Heterozyg-ous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[=] <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=]	Normal
P2.2	32	F	TJP2 [NM_004817.3]:c.[3334C>T];[3334=]	Heterozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[=] <i>ABCG5</i> [NM_022436.2]:c.[148C>T];[=]	Mild cholestasis in pregnancy
P2.8	16	M	TJP2 [NM_004817.3]:c.[3334C>T];[=]	Heterozygous	<i>ABCB11</i> [NM_003742.2]:c.[1331T>C];[1331T=] <sup>1</sup>	Normal

P2.3	31	M	TJP2 [NM_004817.3]:c.[3334=];[3334=]	Wild type	ABCG5[NM_022436.2]:c.[148C>T];[=] UGT1A1[NM_000463.2]:c.-53TA[6];[7]	Normal
P2.9	15	M	TJP2 [NM_004817.3]:c.[3334=];[3334=]	Wild type	ABCB11[NM_003742.2]:c.[1331T>C];[1331T>C] <sup>2</sup> ABCC2[NM_000392.3]:c.[3563T>A];[4544G>A] <sup>1</sup>	Normal
P2.10	-	F	-	-	-	Mild cholestasis, 4 pregnancies?
P2.11	-	F	-	-	-	Mild cholestasis, 3 pregnancies?
P2.12	-	F	-	-	-	Mild cholestasis, 2 pregnancies?

<sup>1</sup>Compound heterozygosity for the *ABCC2* gene variant coding for multidrug resistance protein 2 (MRP2) may be associated with pruritus in patients with primary biliary cholangitis.

<sup>2</sup>Homozygosity for *ABCB11* gene variant coding for bile salt export pump (BSEP) protein is associated with cholestasis in pregnancy and drug-induced cholestasis. M: Male; F: Female.

## DISCUSSION

In this case report, we present a novel variant in the *TJP2* gene in a large family of consanguineous parents using a gene panel approach. This *TJP2* variant caused liver disease in the homozygous state, while heterozygous individuals were unaffected except for a high rate of ICP in pregnancies. In contrast to previously published case reports, our family presented with liver disease and HCC during adolescence or in adulthood. For *TJP2* and for this variant in particular, there seems to be an age-dependent and high penetrance, including increased risk of liver cancer. In addition to this novel *TJP2* variant, the family also harbored variants in other genes involved in cholestatic liver diseases, which may explain differences in genotype-phenotype presentations.

The novel *TJP2* variant [NM\_004817.3]:c.[3334C>T];[3334C>T] (p.(Gln1112\*)) is a nonsense-variant that likely induces nonsense-mediated decay and degradation of the *TJP2*-protein and therefore mimics *TJP2* haploinsufficiency. *TJP2* haploinsufficiency may present with different phenotypes, including typical PFIC4 with rapid progression of cholestatic liver disease mainly in children<sup>[6]</sup>, or unexplained cholestasis with pruritus; with some extrahepatic features as hearing loss<sup>[10]</sup>, or as ICP<sup>[11]</sup>. Previously described *TJP2* variants are also most likely protein truncating with alteration of the reading frame resulting in generation of premature stop codons. This is followed by the absence of the protein, as demonstrated by both Western ligand blotting and immunohistochemistry<sup>[6]</sup>. Normally, the *TJP2* protein binds to junctional transmembrane proteins on the C-terminus of cytoplasmic domains and belongs to a family of membrane-associated guanylate kinase homologs<sup>[12]</sup>. The *TJP2* protein participates in functional junctions in epithelial and endothelial cells<sup>[13]</sup>, where it plays an important role in orientating parts of these paracellular structures interacting with cytoskeletal proteins and integral membrane proteins<sup>[4,6]</sup>. In the absence of *TJP2*, the normal compactness of the tight junctions is impaired, resulting in leakage of bile and biliary substances into the liver parenchyma, which may elicit an inflammatory response, including macrophage activation and fibrosis formation.

In previous studies, several variants have been described in *TJP2* and associated with liver as well as other diseases. We performed a literature search for publications regarding *TJP2* variants as presented in Table 3 with genetic, clinical, biochemical, and histological data. The majority of studies report severe liver disease in children homozygous for a variant in *TJP2*. These children have elevated liver enzymes and bile acids and congenital hepatic fibrosis at age 0-12 years, and phenotypes also include HCC development and need for liver transplantation<sup>[6,14-17]</sup>. Children with the variant in a heterozygous state presented with normal or slightly elevated liver enzymes, bile acids and normal FibroScans<sup>[2,18]</sup>. Regarding other diseases, Wang *et al*<sup>[10]</sup> described autosomal dominant nonsyndromic hearing impairment (ADNSHHI) at the age of 21-68 years in patients with a missense variant in *TJP2* (c.2081G>A (p.G694E)). Carlton *et al*<sup>[19]</sup> described familial hypercholesterolemia age of <1 to 8 years

in children homozygous for TJP2 variants.

Intrahepatic cholestasis of pregnancy (ICP) and stillbirth have been described in women heterozygous for another variant in the *TJP2* gene<sup>[2,11]</sup>. Similarly, the women in the present family heterozygous or homozygous for the TJP2 variant presented with mild and severe ICP, respectively, and the latter needed medical treatment of pruritus and induction of labor (week 38).

In contrast to previous reports on TJP2 variants, homozygous members of this family presented with liver disease in late adolescence or adulthood, including cirrhosis at age 19 (P2.5) and cirrhosis and ACLF at age 35 (P2.1). Two other homozygous brothers, age 23 and 19 (P2.4, P27) and homozygous sister age 21 (P2.6), have only had mild disease with no fibrosis on liver biopsies thus far. However, they all have high FibroScans and sCD163 levels as a marker of macrophage activation involved in liver inflammation and fibrosis.

The basic pathological sign in intrahepatic cholestasis is capillary bile duct occlusion with accumulation of bile in capillary bile ducts, along with zone 3 hepatocytes and Kupffer cells accompanied by hepatocyte degeneration, foam cells, fibrosis formation, and finally cirrhosis<sup>[20]</sup>. In children with early stage PFIC4, capillary bile duct cholestasis and mild hepatocyte degeneration can be observed with hepatocyte giant cell transformation (more common in infancy, gradually recovering with age) and ballooning of hepatocytes. Infant biliary tract injury is mild, but with aging, biliary tract injury progress is accompanied by bile duct paucity. As described above, macrophages seem to be involved in the progression of liver disease with fibrosis and cirrhosis. Macrophage activation can be determined by sCD163 levels, and we have previously demonstrated elevated levels in relation to liver disease severity (*e.g.*, inflammation, fibrosis, portal hypertension) and prognosis<sup>[21,22]</sup>. In support of this, we also showed elevated sCD163 levels in this PFIC4 family in patients with severe liver disease and carrying the variant in a homozygous state compared to family members heterozygous for the variant or wild-type subjects with normal liver parameters. We propose that sCD163 levels and FibroScan in addition to standard biochemistry (alkaline phosphatase, bilirubin, ALAT, INR, GGT, and bile acids) can be used as follow-up in these patients prospectively. In support of this, sCD163 and FibroScan values were especially high in patients with cirrhosis.

Since we used a gene panel approach for the genetic investigation in this family, we discovered some other genetic variants in addition to the TJP2 variant, as shown in [Table 2](#). Of interest in relation to liver diseases were variants in the *ABCB11*, *ABCG5*, *ABCC2* and the *UGT1A1* genes.

Variants in *ABCB11* encoding the BSEP protein result in a variety of cholestatic diseases, such as PFIC2, benign recurrent intrahepatic cholestasis type 2 (BRIC2), drug-induced cholestasis, and intrahepatic cholestasis of pregnancy<sup>[11,23]</sup>. There are some commonly known characteristics of progressive familial intrahepatic cholestasis associated with different genes, as shown<sup>[24,25]</sup> in [Table 4](#). Of the nine tested siblings, the two TJP2-homozygous patients with liver cirrhosis were heterozygous (P2.1) and homozygous (P2.5) for a common disease-associated polymorphism *ABCB11*, respectively; the latter also presented with HCC. This might contribute to the phenotype suggesting that homozygosity for variants in both *TJP2* and *ABCB11* could be a significant risk factor for severe liver disease, including HCC development. Similarly, of the two female siblings presenting with mild (P2.2) and severe (P2.6) ICP, the latter was homozygous for the variants in both *TJP2* and *ABCB11*. This patient may also be at increased risk for HCC, and this warrants close follow-up and monitoring, especially if progression to cirrhosis is observed.

The *ABCC2* (ATP Binding Cassette Subfamily C Member 2) gene is associated with Dubin-Johnson syndrome<sup>[26]</sup> and pruritus in patients with biliary cirrhosis<sup>[27]</sup>. In this family, the father (P1.1) and six siblings harbored two *ABCC2* variants on the same allele, and this combination was associated with more severe liver disease, as indicated in the patients with cirrhosis (P2.1 and P2.5); however, younger patients were less affected apart from ICP (P2.6).

## CONCLUSION

In conclusion, we report an adult PFIC4 family with a novel variant in the *TJP2* gene (TJP2 [NM\_004817.3]:c.[3334C>T];[3334C>T] p.(Gln1112\*)). This variant has not been previously linked to liver disease. In the same family, patients homozygous or heterozygous for this TJP2 variant have different manifestations and severity of disease, which may be explained by age-dependent penetrance of disease and/or the other genetic variants detected by the gene panel approach (*e.g.*, *ABCB11* and *ABCC2* variants). This novel TJP2 variant is associated with adult PFIC4 liver disease with the

Table 3 Literature review of findings of genetically verified tight junction protein 2 variants

Ref.	TJP2 gene	Age	Liver enzymes	Bile acids	Fibrosis	Pruritus	ICP	HCC	Liver failure	TJP2 mutation	TJP2/Included
Vitale <i>et al</i> <sup>[2]</sup>	Heterozygous	37-51 yr	Elevated	Elevated	Normal FibroScan	1 with obvious symptoms	1 with DIC and ICP	NK	NK	p.[T62M]; [p.[I875T]]; [p.[I875T]]	10/48
Sambrotta <i>et al</i> <sup>[6]</sup>	12 children Homozygous	1-3 mo	Low or normal	Elevated	Cirrhosis and portal hypertension on age 4 and 7	No	No	Not described	9 liver transplantations 1.5-4 years age 1 died 13 months	c.766_769del elGCCT, c.885delC c.782delA, c.1361delC, c.1992-2A>G, c.953-735_2356-249del c.3408-?_3573+?del and c.1894C>T	12/12
Wang <i>et al</i> <sup>[10]</sup>		21-68 yr	NK	NK	NK	NK	NK	NK	NK	c.2081G>A(p.G694E)	8/21 Hearing loss
Dixon <i>et al</i> <sup>[11]</sup>	Heterozygous	NK	Elevated	Elevated	NK	Yes	All confirmed ICP 1 stillbirth	NK	NK	p.Thr62Met; p.Thr626Ser c.1877C>G p.Thr626Aer	3/26
Ge <i>et al</i> <sup>[14]</sup>	Compound heterozygous	23 mo	Elevated	Elevated	NK	Alleviated after treatment	-	NK	NK	c.2448 + 1G > C c.2639delC (p.T880Sfs*12)	1/1
Zhou <i>et al</i> <sup>[15]</sup>	Compound heterozygous	26 mo	Elevated	NK	Both with cirrhosis	NK	No	Yes	Yes	2668-1G>T / c.2438dup T (p.Asn814Glnfs)	2/2
Vij <i>et al</i> <sup>[16]</sup>	Homozygous	6 mo	Elevated					Yes, age 2 years	NK	c.817delG (p.A273fs)	
	Homozygous	7 yr	Normal	Elevated	Cirrhotic explant liver and high-grade dysplastic nodule	Yes	No	Yes, early well-differentiated	No	c.(2659+1_2660-1)/(2760+1_2761-1)	1/1
Shagrani <i>et al</i> <sup>[17]</sup>	Heterozygous Homozygous	0-12 yr	Elevated	Normal	1 with congenital hepatic fibrosis	No	No	No	NK But 4 patients required liver transplantation	c.2038delA: p.R680fs c.1012C>T: p.R338X c.1012C>T: p.R338X c.1013delG: p.R338fs c.1190C>T: p.P397L c.1373delC: p.A458fs c.1373delC: p.A458fs	12/37
Chen <i>et al</i> <sup>[18]</sup>	Heterozygous	> 1 yr	NK	Elevated but no detailed data	NK	No	No	No	NK	c.2174G>A; c.343A>G/ c.1377T>G c.343A>G/ c.1377T>G	4/33

Carlton <i>et al.</i> <sup>[19]</sup>	Homozygous	1-8 yr	Almost normal	Almost normal or NK	NK	Yes	NK	NK	NK	c.925C>T 143C/143C	11 individuals with familial hypercholanemia in 8 families
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development of liver cirrhosis and HCC. Patients who are homozygous for this variant should have close follow-up and monitoring of liver disease progression, including HCC screening by ultrasound, standard biochemistry, macrophage activation marker sCD163, and FibroScan.



**Table 4 Common known phenotypes of the genes included in the panel including characteristics of progressive familial intrahepatic cholestasis<sup>[24,25]</sup>**

Mutated gene (deficiency)	Disease association (autosomal recessive)	Disease association (autosomal dominant)
ATP8B1(FIC1)	PFIC1(2009) BRIC1	ICP
ABCB11(BSEP)	PFIC2(2009) BRIC2	ICP Drug-induced cholestasis
ABCB4 (MDR3)	PFIC3	ICP LPAC Drug-induced cholestasis
TJP2(TJP2)	PFIC4	ICP
ABCC2(MRP2)	Dubin-Johnson	ICP
ABCG5(ABCG5)	Sitosterolemia	Cholelithiasis
UGT1A1(UGT1A1)	Crigler-Najjar syndrome type 1; Crigler-Najjar syndrome type 2; Gilbert syndrome (promotor regions)	
NR1H4(FXR)	Intralobular cholestasis	Posttransplant hepatic steatosis
MYO5B(MYO5B)	Microvillus inclusion disease (MVID); Cholestasis without MVID	

ICP: Intrahepatic cholestasis during pregnancy; LPAC: Low phospholipid-associated cholelithiasis; PFIC: Progressive familial intrahepatic cholestasis; BRIC: Benign recurrent intrahepatic cholestasis.

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