Journal of Pediatric Gastroenterology and Nutrition, Publish Ahead of Print

DOI : 10.1097/MPG.0000000000003110

# Progressive Familial Intrahepatic Cholestasis associated with USP53 Gene Mutation in a Brazilian Child

Gilda Porta MD, PhD[1], Paula SM Rigo MD [1], Adriana Porta MD[1], Renata PS Pugliese MD, PhD[1], Vera LB Danesi MD [1], Eliene Oliveira MD[1], Cristian CV Borges MD[1], Cristiane Ribeiro MD [2], Irene K Miura MD, PhD[1]

1. Pediatric Hepatology, Transplant Unit – H.Sírio Libanês / H.Menino Jesus, S.Paulo, Brazil

2. Department of Pathology – H. Sirio Libanês/H. Menino Jesus, S. Paulo, Brazil

Corresponding Author: Gilda Porta

Rua Adma Jafet, 74 cj 81

01308-050

gildaporta@gmail.com

Tel: 5511996320612

Fax number: 551130816436

**Disclosures:** All the authors declare that they have no conflicts of interest to disclose with respect to this study.

**What is known**

- Pathological variants in ATP8B1, ABCB11, NR1H4, TJP2, MYO5B and FXR are implicated in low-GGT intrahepatic cholestasis.
- In about 20% of children with cholestasis and normal or low serum GGT no etiology is identified.

**What is New**

- Recently USP53 gene mutations associated with low-GGT cholestasis have been identified.
- Cholestasis is transient in most cases, although early fibrosis has been described.
- Hearing disorders and hypocalcemia may be related to USP53 gene mutations.

**Abstract**

A case of low-GGT cholestasis associated to USP53 gene mutation in a Brazilian child is described. Transient jaundice and hypocholia started at age 10 days old. Liver enzymes, total bilirubin and total bile acids were elevated at presentation. During follow-up, he developed cholelithiasis treated with cholecystectomy, and an intracranial hemorrhage resolved with full recovery. At last evaluation at 18 months of age, he was not jaundiced and had normal liver tests, but suffered from moderate pruritus despite treatment with rifampicin and ursodeoxycholic acid. A genetic study revealed novel homozygous mutations c.1687_1688delinsC p.Ser563Profs*25 in the *USP53* gene. His parents carried the same heterozygous mutation in the *USP53* gene.

**Key words:** GGT, cholestasis, pathogenic variant



## Introduction

Cholestatic jaundice affects approximately 1 in 2,500 infants and has multiple etiologies.[1] Familial intrahepatic cholestasis (PFIC) accounts for 9-12% of pediatric cholestasis[2,3,4]. PFIC represents a group of disorders characterized by defective bile excretion, causing a multitude of clinical manifestations with variable severity often beginning in childhood. Different gene mutations have been linked to PFIC in the last few decades, and PFIC is currently divided into six types. Applying exome sequencing, Maddirevula et al.[5] recently described two novel mutations causing defects in ubiquitin specific peptidase 53 (USP53 protein) in children with cholestatic liver disease and low glutamiltranspetidase (GGT). Zhang J, et al., discovered pathogenic variants of *USP53* in 7 patients from 7 unrelated families, which were associated with cholestasis.[6]

We report a Brazilian child with normal GGT associated with neonatal cholestasis and a genetic study revealing a very rare PFIC type, not previously reported in South America.

## Case report

A four-month-old male child presented with cholestasis at 10 days of life, with jaundice, fecal hypocolia, and coluria. He was born via Cesarean section at a gestational age of 39 weeks with birth weight of 3340 g. Apgar score was 7/9. Parents were not consanguineous. Routine newborn hearing screening test results were normal. Four paternal relatives had intrahepatic cholestasis with severe pruritus, which had improved with rifampicin, but recurred with the withdrawal of the medication. Viral and other metabolic liver diseases were excluded. Physical examination revealed jaundice and hepatomegaly. Laboratory data showed normal blood count, blood urea nitrogen, creatinine, and electrolytes. Serum bile acid levels were 216

mg/dL (ULN: 10); AST 130 IU/L (ULN: 30); ALT 69 IU/L (ULN: 35 ); GGT 37 IU/L

(ULN: 19 IU/L); total bilirubin 8.98 mg/dL; direct bilirubin 6.69 mg/dL; total cholesterol level 271 mg/dL; INR 1.71; serum calcium 9.3 mg/dL ( 8,5-10,5), and albumin 3.4 g/dl. Liver ultrasound revealed homogeneous hepatomegaly and cholelithiasis. Liver biopsy at 5 months of age showed marked cholestasis, giant cell transformation, portal septal fibrosis, and a periportal ductular proliferation (Figure 1). A cholestatic gene panel revealed novel homozygous mutations c.1687_1688delinsC p.Ser563Profs*25 in the *USP53* gene. The parents carried the same heterozygous mutation in the *USP53* gene. During follow-up, the child developed hemorrhagic stroke, and arteriovenous malformation was suspected. Computed tomography revealed an intracranial hemorrhage. The patient fully recovered with clinical treatment, without sequelae, and a cholecystectomy was performed. After hospital discharge, the cholestasis improved. At last follow up, he had normal length and weight for age (18 months), no jaundice, moderate pruritus, and normal transaminases, on treatment with ursodeoxycholic acid and rifampicin.

**Discussion**

In humans, *USP53* encodes a protein ubiquitin carboxyl-terminal hydrolase 53 (USP53) that is crucial in physiological functions. Our patient, a Brazilian child, not from Arabic or Chinese descent, had neonatal cholestasis with elevated serum bile acid levels, low GGT, and normal serum calcium. Cholestatic gene panel test identified homozygosity for another novel defect in the USP53 gene. Mutations in this gene are associated with progressive hearing loss and cholestasis. Animal models have shown that *USP53* is a component of the tight junction complex.[7] In a mouse-deafness model, tight-junction- associated proteins are involved in the survival of auditory hair cells and hearing, modulating the barrier properties and mechanical stability of tight junctions. Biallelic variants of TJP2 are implicated in hearing impairment [8] and low-GGT intrahepatic cholestasis. Primary loss of the P4-ATPase ATP8B1, known to cause PFIC1, is also associated with deafness. Zhang et al suggested that USP53 mutation may cause a partial phenocopy of TJP2 disease in both liver and ear. They reported seven patients with USP53 disease who also had heterozygous mutations in other genes associated to cholestasis such as *TJP2*, *MYO5B* and *VPS33B* in 3 patients. The importance of these findings warrants further investigation.

The clinical characteristics of the 11 patients, previously reported, with normal or low GGT cholestasis and USP53 gene mutations, including the present case, are shown in the *Table 1*. All patients developed cholestasis before 7 months of age, and cholestasis was transient in 8 patients (73%). Hearing disorders were observed in 3 patients, one of them received cochlear implant. Hypocalcemia was present in all 3 Saudi children. The medications prescribed were UDCA, cholestyramine and rifampicin. One case (from Saudi Arabia) was transplanted at 6 years-old due to intractable pruritus. There was One lost of follow-up. In the present case, moderate itching persists, despite the use of UDCA and rifampicin. None of the patients died. Excluding the case of loss of follow up, none of patients remained jaundice, and the transaminases were completely normal in 7 cases.

Liver biopsy was performed in 4 chinese patients, and showed intralobular cholestasis and fibrosis in all. The 3 patients from Saudi Arabia were from the same family (two sisters and a cousin), hence carried the same pathogenic variant c.951del:p. (Phe317Leufs*6). In the Zhang series, 2 known and 8 novel pathogenic variants were described - the 2 previously reported variants were c.1012C>T (p.Arg338Ter) and c.1426C>T (p.Arg476Ter), and the 8 novel variants included 5 that are undoubtedly pathogenic – c.569+2T>C; c.169C>T (p.Arg57Ter); c.581delA (p.Arg195GlufsTer38); c831_832insAG (p.Val279GlufsTer17) and c.1558C>T (p.Arg520Ter); three were likely pathogenic: c.297G>T (p.Arg99Ser); c.395A>G (p.His132Arg) and c.878G>T (p.Gly293Val). In the present case, we describe a new homozygous mutation in the USP53 gene: c.1687_1688delinsC p.Ser563Profs*25.

In conclusion, defects in *USP53* can be considered a new genetic cause of low-GGT cholestatic liver disease, beginning early in life, which can occur in different ethnicities and may be associated with several genetic variants. Cholestasis seems to be transient, but liver fibrosis can appear early. Hearing loss and hypocalcemia have been described in some cases.

**References**


1. Balistreri WF. Neonatal cholestasis. J Pediatr 1985;106:171–84.
2. Chen HL. Mining the idiopathic genetic cholestasis syndrome. J Gastroenterol Hepatol 2013; 28:389–91.
3. Feldman AG, Sokol RJ. Neonatal cholestasis. Neoreviews 2013;14: e63–73.
4. Spraul-Davit A, Gonzales E, Baussan C, et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Dis 2009;4:1.
5. Maddirevula S, Alhebbi H, Alqahtani A, et al. Identification of novel loci for pediatric cholestatic liver disease defined by KIF12, PPM1F, USP53, LSR, and WDR83OS pathogenic variants. Genet Med 2019;21:1164-72.
6. Zhang J , Yang Y, Gong JY, et al. Low GGT intrahepatic cholestasis associated with biallelic USP53 variants: histological, and ultrastructural characterization. Liver Int 2020;40:1142-50.
7. Heinemamm U, Schuetz A. Structural features of tight-junction proteins. Int J Mol Sci 2019; 20: 6020.
8. Kazmierczak M, Harris SL, Kazmierczak P, et al. Progressive hearing loss in mice carrying a mutation in Usp53. J Neurosci 2015; 35: 15582-98.

Figure 1: Cholestasis, giant cell transformation, portal septal fibrosis, and a periportal ductular proliferation

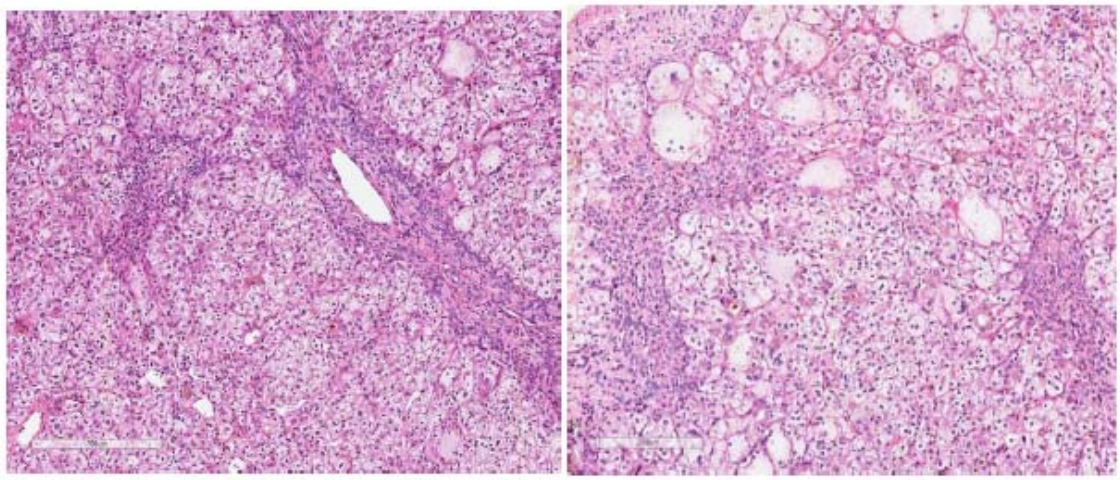

Table 1: Characteristics of patients with normal or low-GGT cholestasis associated to USP53 gene mutations

| Author | PATIENT | GENDER | AGE | DEAFNESS | HYPOCALCEMIA | MEDICATIONS | AST/ALT | ALP/GGT | TB/DB | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| Maddirevula et al, 2019* | 1 | Female | 4 mo | yes | yes | nr# | 97/82 | 6316/35 | 159/132 | liver transplantation at 6 y. Alive at 23 y |
| | 2 | Female | 5 mo | yes | yes | Rifampicin | 25/29 | 4432/39 | 26/20 | sister of pt 1. Alive at 15 y |
| | 3 | Male | 1 y | no | yes | Rifampicin | 136/352 | 2557/30 | 402/298 | cousin of pt 1 and 2. Alive at 3 y |
| Zhang J et al, 2020** | 4 | Female | 3 d | no | nr# | UDCA and cholestyramine | 215/184 | 330/23 | 23/19 | alive at 2 y |
| | 5 | Male | 2 d | no | nr | UDCA and cholestyramine | 71/70 | 548/72 | 90/65 | alive at 3y6m |
| | 6 | Female | 6 mo | no | nr | UDCA | 121/103 | nr/34 | 212/159 | alive at 5y |
| | 7 | Male | 5 mo | no | nr | UDCA and cholestyramine | 84/32 | 636/39 | 308/167 | alive at 17mo |
| | 8 | Female | 1 mo | no | nr | UDCA | 51/28 | 543/40 | 275/216 | lost to follow-up |
| | 9 | Male | 5 mo | yes | nr | UDCA and cholestyramine | 41/26 | 342/27 | 85/72 | alivewith 1y3mo; cochlear implant at 1y3mo |
| | 10 | Male | 7 mo | no | nr | UDCA and cholestyramine | 225/18 | 283/22 | 153/137 | alive at 1y1mo |
| Porta et al, 2020*** | 11 | Male | 10 d | no | no | UDCA and rifampicin | 130/69 | 579/37 | 153/114 | alive at 18 m with moderate itching |

# nr=not reported
*Reference values AST/ALT 0-40 U/L; ALP 250-350 U/L; GGT 0-30 U/L; TB/DB 5-17 mmol/l
**Reference values ALT 0-40 IU/L; AST15-60 IU/L; ALP 42-383 IU/L; GGT 7-50 IU/L; TB 5.1-20 mmol/l; DB 0-6 mmol/l
*** Reference value upper limit of normal ALT 35 IU/L; AST 30 IU/L; ALP 390 IU/L; GGT 30 IU/L; TB reference values 0.6-1.4 mg/dl; DB reference values 0-0.6 mg/dl