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Case report

Alagille syndrome caused by *NOTCH2* mutation presented atypical pathological changes

Yiling ShenTu [a,1], Xiaoxiao Mi [b,1], Dong Tang [c], Yanming Jiang [a], Ling Gao [a], Xiaojie Ma [a], Bing Zhou [d], Wenjun Yang [e], Junping Shi [a], Dixiang Lan [f], Gongying Chen [a], Ling Gong [a,*]

[a] Department of Infectious Disease (Liver Diseases), The Affiliated Hospital of Hangzhou Normal University, Zhejiang, China
[b] Institute of Translational Medicine, The Affiliated Hospital of Hangzhou Normal University, Zhejiang, China
[c] Department of Medical Imaging (Radiology), The Affiliated Hospital of Hangzhou Normal University, Zhejiang, China
[d] Department of Interventional Vascular, The Affiliated Hospital of Hangzhou Normal University, Zhejiang, China
[e] Department of Pathology, The Affiliated Hospital of Hangzhou Normal University, Zhejiang, China
[f] Department of Infectious Disease, The First People's Hospital of Yuhang District, Zhejiang, China

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ABSTRACT

Background: Alagille syndrome (ALGS) is a rare multisystem disorder caused by mutations in the *JAG1* or *NOTCH2* gene. However *NOTCH2* gene mutations were rarely found in the Alagille patients. Little is known about the clinical and pathological profiles about Alagille patients with *NOTCH2* mutation.
Case report: Our case described a 16-year-old female patient manifesting as recurrent jaundice and abnormal liver function from the second week of her birth. She presented a butterfly vertebrae and typical facial features including a prominent forehead, deep-set eyes, a pointed chin, and a straight nose with bulbous tip. Pathogenic heterozygous c.5857 C > T variant in *NOTCH2* gene was found. Her liver biopsy featured by a disorder liver structure with cholestasis and fibrosis in portal area, which is different from typical bile duct paucity reported in *JAG1* deficient patients.
Results: A diagnosis of ALGS was made. The patient was treated with ursodeoxycholic acid and compound embryonic bovine liver extract tablets and infusion of human serum albumin to improve her clinical and pathological symptoms.
Conclusion: Since Alagille patients with *NOTCH2* mutations have been rarely reported, our case will highlight the clinical and pathological profiles of these patients.

1. Introduction

Alagille syndrome (ALGS) is a complex autosomal dominant multisystem disorder with an incidence of 1:30,000 per live births [1,2]. It is caused by defects in *JAG1* and *NOTCH2*. Up to 97% of reported cases are caused by mutations in *JAG1*, with less than 1% of patients caused by *NOTCH2* mutations [3,4]. Unclear relationship between genotypes and phenotypes, variable penetrance, and the alterations in clinical presentations both increase the difficulty in the diagnosis of ALGS syndrome with *NOTCH2* mutations [5]. Clinical features of *NOTCH2*-related ALGS are not as typical as that of *JAG1*-related ALGS [6]. Over 500 cases of ALGS have been reported since its initial reported [7], but a few cases with *NOTCH2* mutations have been reported. Therefore, it is necessary to understand clinical manifestations and pathological profiles in ALGS patients with *NOTCH2* mutations.

We present here a female patient diagnosed as ALGS who carries a pathological *NOTCH2* gene variant (c.5857 C > T, p.Arg1953Cys). We also present a detail description of her clinical and pathological profiles. Our case will provide good reference for diagnosis of *NOTCH2*-related ALGS patients in the future.

Abbreviations: ALGS, Alagille syndrome; *JAG1*, *Jagged1*; TBIL, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AKP, alkaline phosphatase; GGT, γ-glutamyl transferase; DBIL, direct bilirubin; TBA, total bile acid; ALB, albumin; MRI, magnetic resonance imaging; MRCP, magnetic resonance cholangiopancreatography.

* Corresponding author at: Department of Infectious Disease (Liver Diseases), The Affiliated Hospital of Hangzhou Normal University, 310015 Zhejiang, China.
E-mail address: gongllyy22@163.com (L. Gong).

[1] Yiling ShenTu and Xiaoxiao Mi contributed equally.

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2. Case presentation

A 16-year-old female patient was admitted to our hospital with recurrent jaundice and abnormal liver function since her second week of birth.

She developed jaundice with hepatomegaly and splenomegaly at the time of her second week of birth. As she stated, she recovered gradually after taking an unclear medicine and some kinds of Chinese traditional medicine at that time. However, at her age of 12, she appeared with jaundice again with a high level of total bilirubin and elevated levels of liver enzymes. She was treated with ursodeoxycholic acid and Chinese traditional medicine since then. However, her liver function was not improved (Fig. 1, A–C). She was referred to our hospital at her age of 16.

On admission, special facial features were identified, including a broad forehead, bulbous tip of the nose, a pointed chin, and a characteristic triangular appearance (Fig. 1, D).

Her biochemical tests showed elevated levels of total bilirubin (TBIL, 42.1 μmol/L, reference range: 0–23 μmol/L), direct bilirubin (DBIL, 9.3 μmol/L, reference range: 0–4 μmol/L), alanine transaminase (ALT, 42 U/L, reference range: 0–40 U/L), aspartate aminotransferase (AST, 53 U/L, reference range: 0–40 U/L), glutamyl transpeptidase (GGT, 96 U/L, reference range: 7–45 U/L), total bile acid (TBA, 25 μmol/L, reference range: 0–10 μmol/L), alkaline phosphatase (AKP, 137 U/L, reference range: 30–120 U/L) and a decreased level of albumin (ALB, 36.4 g/L, reference range: 40–55 g/L). Blood routine tests showed leukopenia and thrombocytopenia, and blood coagulation function was abnormal (PT: 16.9 s, reference range: 9.5–12.2 s; INR: 1.57, reference range: 0.80–1.20). Humoral immunity was basically normal. Autoantibody tests showed antinuclear antibody was positive (1:40, reference range: negative), and other auto-antibodies were negative. Viral hepatitis tests were negative.

Abdominal ultrasound showed signs of liver cirrhosis and splenomegaly (data not shown). MRI images of upper-abdomen showed liver cirrhosis with volume atrophy, liver minerals deposition, splenomegaly, multiple tortuous widening vascular in the hilum of spleen, and local splenic-renal venous shunt (Fig. 2, A–B). MRCP manifested as both abnormal morphological and disordered structure of intrahepatic and extrahepatic bile duct (Fig. 2, C). Skeleton images revealed an irregular vertebra at T10 present as an incomplete butterfly vertebra (Fig. 2, D–E) and multiple Schmorl's nodes (Fig. 2, F). Both eye examination and echocardiography revealed no obvious abnormalities.

HE staining of her liver biopsy revealed a disordered lobular structure with cholestasis and fibrosis, with loss of hepatocytes but bile ducts and vascular structure remained. The enlarged fibrosis portal area and inflammatory cell infiltration with mild interfacial inflammation were also observed (Fig. 3, A–B). CK8/18 staining showed fibrosis in portal area and deletion of hepatocytes in large areas (Fig. 3, C). Orcein staining showed Mallory's bodies and balloon-like hepatocytes (Fig. 3, D). CK7 and CD10 staining also showed bile ducts were present and hepatocytes were lacked in large areas (Fig. 3, E–F).

A genetic screening panel was applied to screen gene alterations in this patient. A heterozygous variation in exon11 of *ATP7B* gene (c.2621 C > T, p.Ala874Val) and a heterozygous mutation in exon32 of the *NOTCH2* gene (c.5857 C > T, p.Arg1953Cys) were found (Fig. 4, A–B). Variants in *JAG1* gene were not found (data not shown). Since the patient does not present any manifestations of Wilson's disease, so we focus on the variant found in *NOTCH2* gene. Sanger sequencing of the targeted variation was performed in the patient's family members (Fig. 4, C-E). Her mother and brother displayed a wild-type genotype of targeted *NOTCH2* variant. The structure analysis of the mutant was preformed (Fig. 4, F). Her father died ten years ago in a traffic accident, and we could not get any genetic information about him. It was described that her father was in good health until he died in a traffic accident.

Collectively, based on the molecular diagnosis results and clinical features and pathological changes, a diagnosis of ALGS was made. The

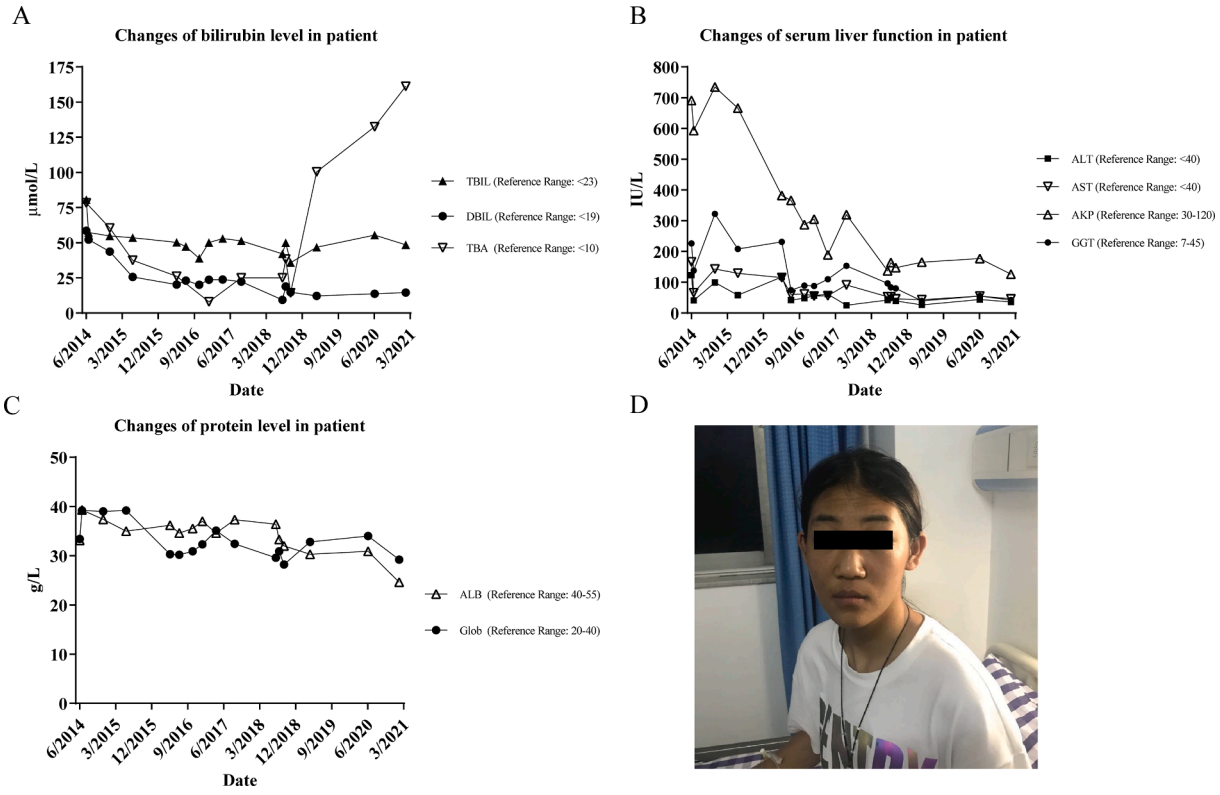

Fig. 1. Serum liver function changes and facial features of the patient. (A) Serum TBIL, DBIL, and TBA changes of the patient. (B) Serum ALT, AST, AKP, and GGT changes of the patient. (C) Serum ALB and Glob changes of the patient. (D) The patient had ALGS-related facial features including a broad forehead, hypertelorism with deep-set eyes, bulbous tip of the nose, and a pointed chin.

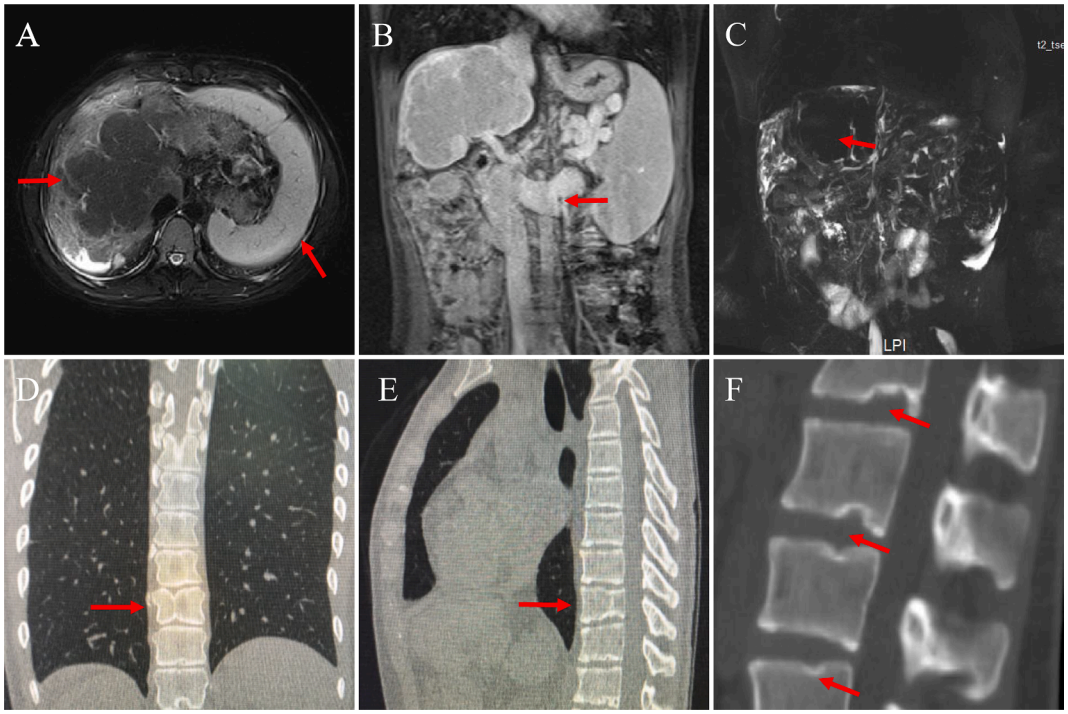

Fig. 2. Imaging and spinal findings of the patient. (A) MRI of upper abdomen showed splenomegaly and atrophy of hepatic lobe with wavy surface changes (arrows). (B) Splenorenal shunt was observed (arrow). (C) MRCP indicated significant sparse distribution and disordered structure of intrahepatic bile ducts (arrow); (D–F) Spinal images. All images are two-dimensional reconstruction of CT images. T-Spine images (D, E) showed an incomplete butterfly vertebra at T10 (arrows). L-spine images (F) shows multiple focal depressions at the adjacent edge of lumbar vertebra, known as "Schmorl's node" (arrows).

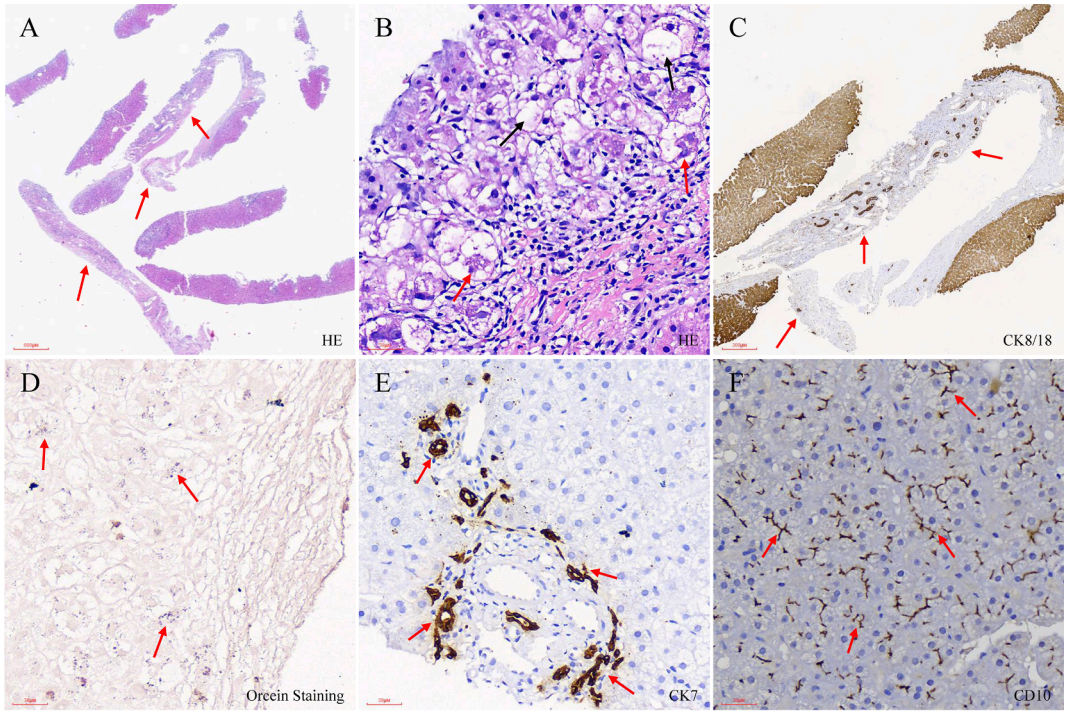

Fig. 3. Liver biopsy. (A-B) HE staining of liver biopsy showed disorder liver structure with fibrosis (A, arrows), Mallory's bodies and balloon-like hepatocytes (B, arrows). (C) CK8/18 staining showed fibrosis in portal area and deletion of hepatocytes in large areas (arrows). (D) Orcein staining showed Cu deposition positive after cholestasis. (E) CK7 staining showed bile ducts (arrows). (F) CD10 staining showed bile ducts (arrows).

patient was treated on ursodeoxycholic acid to help improve cholestasis, compound embryonic bovine liver extract tablets to treat hepatic fibrosis, and infusion of human serum albumin to improve hypoalbuminemia.

3. Discussion

Alagille syndrome is a rare autosomal dominant inheritance disease mostly caused by mutations in *JAG1* gene. Since few cases of *NOTCH2*-

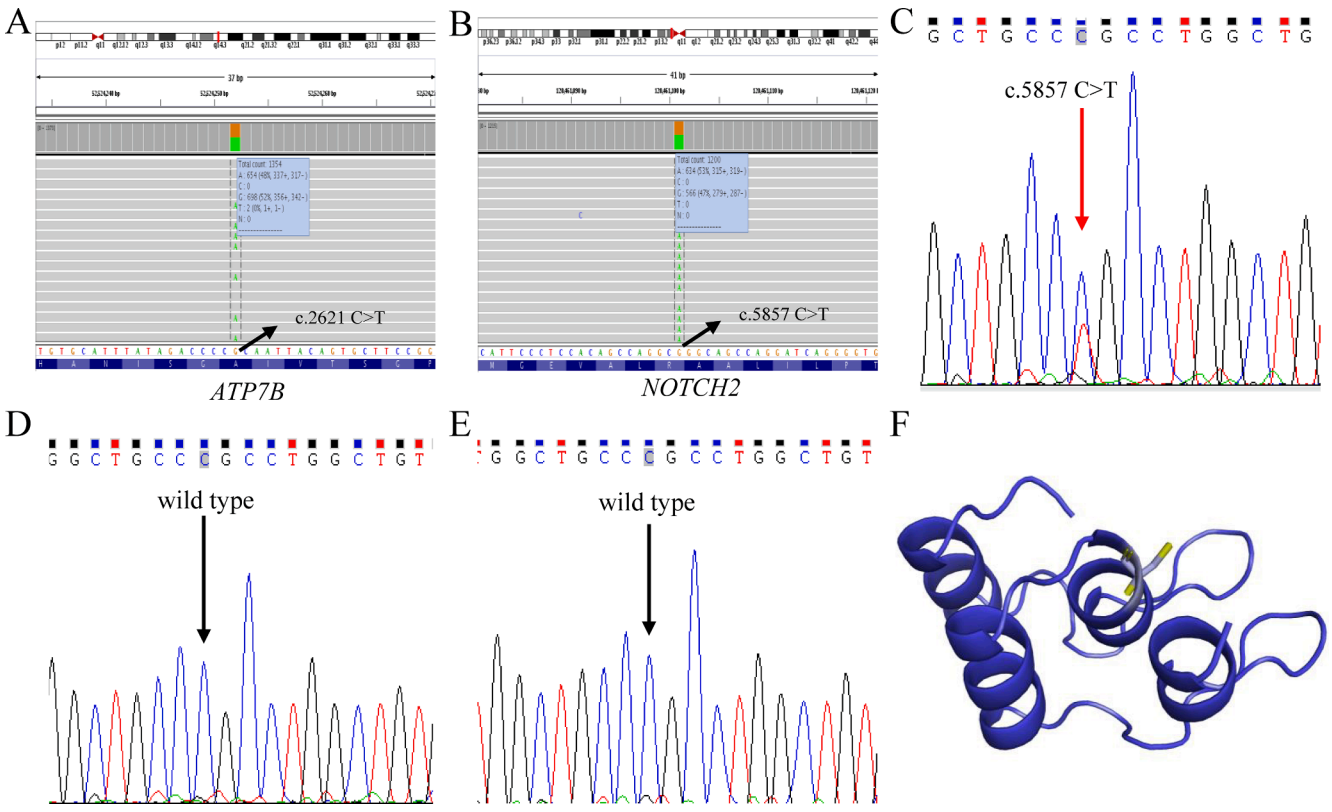

Fig. 4. Results of genetic test. (A) Identification of an *ATP7B* pathogenic variant in the patient. (B) Identification of a *NOTCH2* pathogenic variant in the patient. (C–E) The results of Sanger sequencing of targeted *NOTCH2* variation in her family members. The arrows indicate the alteration from C to T in *NOTCH2* gene of the patient (C). The patient's mother and brother were the wild-type genotype of the *NOTCH2* alterations (D-E). (F) Location of p.Arg1953Cys in *NOTCH2* gene. The structure of *NOTCH2* gene (PDB accession 2004) with the Arg substituted with Cys was performed by PyMol. The difference in length of the Arg and Cys side chains are indicated in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

related Alagille patients were reported, the clinical and pathological presentations are unclear.

In our case, a heterozygous *NOTCH2* mutation was found. There was only one ALGS case reported so far carrying this variant in *NOTCH2* gene. Our patient also carried a heterozygous variant in *ATP7B* gene, we also tried to search the clinical or pathological proofs of Wilson's disease from this patient. Examination result of 24-hour urinary copper was within the normal range. Kayser-Fleischer ring examination was negative. A slightly low level of serum ceruloplasmin (0.12 g/L, reference range: 0.22–0.58 g/L) was found. According to the Leipzig Wilson's disease diagnostic score system [8], the total score of the patient is only 2 points, which indicates a very unlikely diagnosis of Wilson's disease.

A liver biopsy in patients with ALGS typically demonstrates paucity of the bile ducts, however in our patient, several atypically features but paucity of the bile ducts can be found, such as disorder liver tissue structure, loss of hepatocytes in large areas, cholestasis, Mallory's bodies, and balloon-like hepatocytes. Loss of hepatocytes in large areas is not usually seen in ALGS patients, as our patient has a history of liver failure from a child, which may explain her liver tissue phenomenon. It also has been reported that some ALGS patients associated with *NOTCH2* mutations showing no bile duct deficiency [6]. A total of 6 *NOTCH2*-positive individuals who developed cholestasis without other ALGS related clinical features were identified in 2 retrospective case series studies [5,6]. In recent years, liver biopsy is no longer considered requisite to make the diagnosis of ALGS, and the presence of cholestasis is also acceptable to fulfill diagnosis criterion [4,9].

The ALGS-related *NOTCH2* gene mutation was first identified in 2001 by McDaniell *et al.* A *NOTCH2*-deficient mouse model showed ALGS-like symptoms which prompted McDaniell *et al.* to screen *NOTCH2* variations in a cohort which did not have a *JAG1* mutation [10]. Then 2 probands with diverse *NOTCH2* mutations were identified [10,11]. To date, a total of 20 *NOTCH2* mutations-related ALGS cases have been reported [12–14,16,17]. We summary *NOTCH2* mutations reported so far and found that the mutation spectrum is predominantly comprised of missense variations, followed by nonsense, frameshift, and splice site variations. The missense variations accounted for 71.4% (15/21) of all the mutations (Table 1).

Identified *NOTCH2* mutation is not novel but it is already reported in a previous paper [6]. On the one hand there is a lack of novelty but on the other hand this could be a formal proof of the pathogenicity of the mutation and an explanation for a causal role in the disease. The clinical presentation in our patient was not the same compared to the other one described in literature sharing the same mutation. Similar findings in both patients include liver abnormities (cholestasis, portal hypertension, no bile ducts paucity), normal presentation of renal and eye. Moreover, butterfly vertebra, special facial features, and normal finding of cardiac presented in our patient. The other one described in literature had mild pulmonary stenosis with no abnormity either in skeletal or facial features.

Identified *NOTCH2* gene mutation (p.Arg1953Cys) is located in ankyrin repeats 4 of the intracellular domains, which is involved in the NOTCH intracellular domain's binding to DNA to effect gene transcription. In silico analysis of the *NOTCH2* variant using PolyPhen-2 showed this variant is predicted to be probably damaging, with a score of 0.999 (sensitivity: 0.09; specificity: 0.99). The functional effects of amino acid substitutions were also determined through SIFT web servers that predict p.Arg1953Cys variant damage protein function (provean score: -7.504; prediction: deleterious). A three-dimensional structure for *NOTCH2* was constructed using homology modeling by SWISS-MODEL, and was visualized using PyMOL (Fig. 4, F). The amino

Table 1
Clinical and genetic features of probands with *NOTCH2* variants reported.

Proband	Mutation	Type of mutation	Liver	Cardiac	Eye	Skeletal	Facies	Renal	References
1	p.Cys373Arg	Missense	Cholestasis, bile duct paucity	Pulmonary artery stenosis	Posterior embryotoxon	Normal spine	Present	Vesico-ureteric reflux	Kamath et al. [6]
2	p.Pro383Ser	Missense	Elevated LFTs, bile duct paucity	Normal	Posterior embryotoxon	Normal spine	Absent	NA	Kamath et al. [6]
3	p.Pro394Ser	Missense	Cholestasis, bile duct, proliferation, hepatic mass	Atrial septal defect, mild pulmonary stenosis	Posterior embryotoxon	Normal spine	Absent	Normal	Kamath et al. [6]
4	p.Cys444Tyr	Missense	Cholestasis	Tetralogy of Fallot	Posterior embryotoxon	Normal spine	Absent	Renal failure	Mcdaniell et al. [10]
5	p.Cys480Arg	Missense	Bile duct paucity	Normal	NA	Normal spine	Absent	Normal	Kamath et al. [6]
6	p.Ser856fs	Frameshift	Cholestasis, bile duct paucity	Normal	Normal	Normal spine	Absent	Normal	Kamath et al. [6]
7	p. Arg1953Cys	Missense	Cholestasis, portal hypertension	Mild pulmonary stenosis	Normal	Normal spine	Absent	Normal	Kamath et al. [6]
8	p.Arg1953His	Missense	Cholestasis, bile duct paucity	Normal	Cataracts	Normal spine	Absent	Normal	Kamath et al. [6]
9	p.Arg2003*	Nonsense	Cholestasis	Atrial septal defect	NA	Butterfly vertebrae	Absent	Echogenicity of kidneys	Kamath et al. [6]
10	c.5930-1 G > A	Splice site alteration	Cholestasis	Pulmonary stenosis	Normal	Normal spine	Present	Neonatal renal failure	Mcdaniell et al. [10]
11	c.5983-5984delTT	Frameshift	Elevated liver enzymes	Atrial septal defect	NA	Normal spine	Present	Normal	Liu et al. [16]
12†	p.Ser1741Leu	Missense	Cholestasis, bile duct paucity	Normal	Normal	Normal	Present	NA	Vilarinho et al. [17]
12	p.His1882Tyr	Missense	–						Vilarinho et al. [17]
13	p. Met2042Thr	Missense	Cholestasis	Pulmonary arterial hypertension	NA	NA	Present	NA	Zheng et al. [13]
14	p.Cys286Ser	Missense	Cholestasis, bile duct paucity	Normal	Normal	NA	Present	NA	Pacheco et al. [14]
15	p.Arg1933*	Nonsense	NA						Gilbert et al. [12]
16	p.Gln1811*	Nonsense	NA						Gilbert et al. [12]
17	p.Aln1920Pro	Missense	NA						Gilbert et al. [12]
18	p.Asp473Val	Missense	NA						Gilbert et al. [12]
19	p.Asp473Gly	Missense	NA						Gilbert et al. [12]
20	p. Gln1656Arg	Missense	NA						Gilbert et al. [12]

Notes: NA, not available; †Proband 12 had compound heterozygous variants of *NOTCH2* gene.

acid sequence of *NOTCH2* (UniProt: Q04721) was obtained from the UniProt (https://sparql.uniprot.org/). In the protein structure, substitution of Arg with Cys reduces the bulk of the side chain and could be deleterious.

Also of note, individuals carrying mutations in *NOTCH2* gene have a lower penetrance of clinical features compared with those carrying *JAG1* mutations. Skeletal anomalies and facial features were infrequently found in *NOTCH2* mutation-related individuals. The penetrance of *NOTCH2*-mutations is low and highly variable, ranging from apparently normal phenotype to severe liver failure [15]. Some patients display serious clinical manifestations from birth or early infancy. Some patients might suffer from apparently isolated cholestasis of unknown cause or even have only a particular ocular malformation [2]. The relationship between genotype and phenotype in *NOTCH2* mutation-related individuals required further investigation.

We suggested a routine blood test and liver imaging for the patient. After two and a half years of treatment, her liver function improved.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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