Original Article

Clinical and genetic characteristics of patients with Alagille syndrome in China: identification of six novel *JAG1* and *NOTCH2* mutations

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Background: Alagille syndrome (ALGS) is a rare disease. The variable clinical manifestations make the diagnosis of ALGS difficult. This study aimed to provide a basis for the early diagnosis of ALGS patients whose clinical identification is difficult and to enrich the spectrum of genetic variants implicated in Chinese children with ALGS.

Methods: From August 2016 to August 2022, 14 children with ALGS were enrolled in this retrospective study. Clinical and related data were obtained from medical records.

Results: Among the 14 patients, 11 were males and 3 were females. The age of first manifestation of liver disease mean (Q1, Q3) was 0.4 (0.1, 37.0) months, and the age of diagnosis mean (Q1, Q3) was 5.6 (2.4, 48.5) months. Cholestasis was seen in 14 patients, cardiac defects in eight, characteristic facial features in 11, skeletal abnormalities in six, and renal abnormality in one. Among eight patients who underwent ophthalmological examination, posterior embryotoxon was seen in two. We identified 12 different *JAG1* gene mutations and two different *NOTCH2* gene variations. Among the mutations detected, six were novel, including c.2849_2850del (p.S950*), c.35_45delGCCCCCTAAGC (p.R12Pfs*57), c.1860delC (p.F621Sfs*122), and c.1293_1294insTAGTAGACA (p.A432*) in *JAG1*, and c.6040_6041 del (p.L2014Vfs*10) and c.1915+1G>T (splicing) in *NOTCH2*. The follow-up time mean (Q1, Q3) was 48.5 (11.5, 69.0) months; four patients had delayed growth, eight had pruritus, two had xanthomas, seven had elevated bilirubin, and 13 had elevated transaminase. All patients were stable after medical treatment.

Conclusions: ALGS presents a variety of clinical manifestations. Some patients may be misdiagnosed with biliary atresia due to bile duct proliferation in liver biopsies along with biochemical abnormalities. Genetic testing is helpful for early diagnosis. *JAG1* and *NOTCH2* gene mutant spectra are abundant and there are many novel mutations in Chinese children with ALGS.

Keywords: Alagille syndrome (ALGS); *JAG1* gene; *NOTCH2* gene; China; gene mutations

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Introduction

Alagille syndrome (ALGS) is an autosomal dominant disorder characterized by congenital intrahepatic bile duct hypoplasia and cholestasis (1). ALGS was first reported by Alagille in 1969. The incidence rate has been reported at 1:70,000–100,000 among live births, but with the development and application of molecular genetics, the true incidence rate may be close to 1:30,000 (2). The classical diagnostic criteria for ALGS include liver histology showing the paucity of bile ducts and three or more of the following clinical manifestations: chronic cholestasis, cardiac malformations, skeletal abnormalities, ophthalmologic abnormalities, and characteristic facial features (3). In the Global Alagille Alliance (GALA) study, 1,433 children with ALGS from 67 centers in 29 countries were analyzed. The incidence of liver involvement (including a history of neonatal cholestasis, elevated liver aminotransferase, histological abnormalities, history of pruritus and/or xanthomas, or having undergone hepatobiliary surgery) was 95%, that of cardiac abnormalities was 91%, that of specific facial features was 90%, that of posterior embryotoxon was 51%, that of butterfly vertebra was 44%, that of renal abnormalities was 39%, and that of vascular abnormalities was 36% (4). However, some children with ALGS have atypical clinical manifestations, especially small infants who only present with cholestasis, which is easily confused with biliary atresia (BA) and progressive familial intrahepatic cholestasis (PFIC). Genetic testing could help to identify these diseases (5,6).

ALGS is caused by mutations in genes in the intercellular NOTCH signaling pathway, including *JAG1* encoding the JAGGED1 ligand and *NOTCH2* encoding the NOTCH2 receptor (7). The NOTCH signaling pathway plays important roles in the formation and maintenance of bile ducts, cardiovascular development and homeostasis, bone development and remodeling, and kidney development and maintenance (8-11). Guru Murthy *et al.* pointed out the importance of family history, renal abnormalities, and *JAG1* gene mutations in the diagnosis of ALGS (12). As the research moves along, *NOTCH2* mutations should also be considered (2,12,13).

In recent years, although studies on children with ALGS in China have increased (14-21), they remain limited. Therefore, we analyzed the clinical features and genetic pathogenic variants of 14 children with ALGS in our hospital with the aim of better understanding the characteristics of children with ALGS in China and enriching the genetic variation profile. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-24-301/rc).

Highlight box

Key findings

- We identified 12 different *JAG1* gene mutations and two different *NOTCH2* gene variations. Among the mutations detected, six were novel, including c.2849_2850del (p.S950*), c.35_45delGCCCCCTAAGC (p.R12Pfs*57), c.1860delC (p.F621Sfs*122), and c.1293_1294insTAGTAGACA (p.A432*) in *JAG1*, and c.6040_6041 del (p.L2014Vfs*10) and c.1915+1G>T (splicing) in *NOTCH2*.

What is known and what is new?

- Alagille syndrome (ALGS) is congenital intrahepatic bile duct hypoplasia caused by the NOTCH signaling pathway. Early diagnosis of ALGS is difficult.
- Six new mutations have been found in known genes responsible for ALGS (*JAG1* and *NOTCH2*).

What is the implication, and what should change now?

- This study enriches the spectrum of genetic variation in Chinese children with ALGS. Genetic testing is helpful for early diagnosis.
- In subsequent studies, the study population size should be expanded, and multicenter research is further needed.

Methods

Patients

This is a retrospective patients summary. From August 2016 to August 2022, 14 children diagnosed with ALGS in the Children's Hospital, Zhejiang University School of Medicine, were selected as the participants. The inclusion criteria were as follows (3,12-14,22): (I) liver biopsy showed the paucity of bile ducts, and at least three major clinical manifestations (chronic cholestasis, cardiac malformations, skeletal abnormalities, ophthalmologic abnormalities, and characteristic facial features); (II) the absence of the paucity of bile ducts and at least four of six clinical features (chronic cholestasis, cardiac abnormalities, skeletal abnormalities, ophthalmologic abnormalities, characteristic facial features, and renal abnormalities); (III) those who did not meet (I) and (II), but whose genetic testing showed the defects in the *JAG1* gene or the *NOTCH2* gene.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of Children's

Table 1 Clinical characteristics of 14 children with Alagille syndrome

Patient No.	Gender	Age at onset (m)	Age at diagnosis (m)	Cholestasis	Characteristic face	Cardiac abnormalities	Renal dysplasia	Vertebral abnormalities	Posterior embryotoxon
1	Male	0.1	4.8	+	+	PS	–	–	–
2	Male	0.1	50.0	+	+	–	–	+	–
3	Male	0.1	2.7	+	–	VSD	–	–	NA
4	Female	0.1	2.2	+	+	PDA + ASD	–	+	+
5	Male	65.0	68.0	+	+	–	+	+	NA
6	Male	0.5	2.5	+	–	PDA + ASD	–	+	–
7	Male	40.0	51.0	+	+	–	–	+	NA
8	Female	43.0	43.0	+	+	–	–	–	NA
9	Male	0.2	3.5	+	+	VSD	–	–	–
10	Male	1.0	13.0	+	+	–	–	–	–
11	Female	2.5	6.5	+	+	–	–	–	–
12	Male	0.1	2.0	+	–	ASD + VSD	–	–	NA
13	Male	36.0	48.0	+	+	ASD + VSD	–	+	+
14	Male	0.1	1.0	+	+	ASD + PS	–	–	NA

+, positive; –, negative. m, months; PS, pulmonary artery stenosis; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; NA, not available.

Hospital, Zhejiang University School of Medicine (No. 2023-IRB-0168-P-01) and individual consent for this retrospective analysis was waived.

Collection of clinical data

General information, clinical manifestations, physical signs, laboratory examinations, imaging examinations, ophthalmic examinations, liver histopathological examinations, genetic results of the patients, and follow-up information were collected from a computerized hospital database.

Genetic analysis

Peripheral blood samples were obtained from 14 ALGS patients and their parents. Among these patients, nine were subjected to whole-exome sequencing (WES), and five patients were analyzed via multigenic panels related to cholestasis. Additionally, their parents were validated through Sanger sequencing. On the basis of the American College of Medical Genetics and Genomics (ACMG) guidelines, the variants detected were classified into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign (23).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables are expressed as numbers and percentages, whereas normally distributed continuous variables are summarized as the mean ± standard deviation and nonnormally distributed continuous variables are expressed as mean (Q1, Q3).

Results

Clinical manifestations of the patients

The clinical characteristics and related data of the 14 children with ALGS are shown in *Table 1*. Among the 14 children, 11 were males and three were females. The age of first manifestation of liver disease mean (Q1, Q3) was 0.4 (0.1, 37.0) months, and the age of diagnosis mean (Q1, Q3) was 5.6 (2.4, 48.5) months. Among these patients, 14 (100%) developed from cholestasis, whereas four (28.6%) developed from elevated alanine transaminase (ALT) or aspartate transaminase (AST) levels (normal bilirubin).

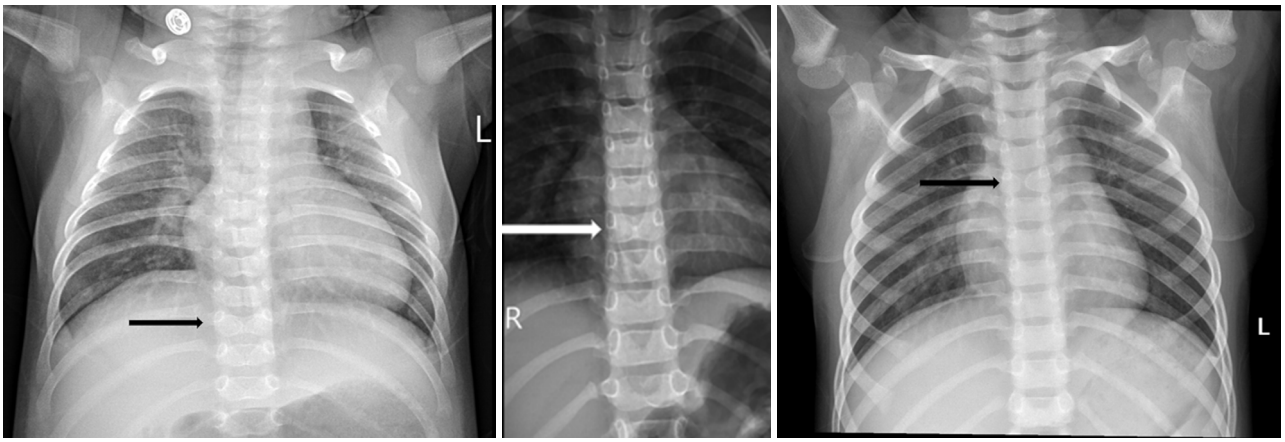

Figure 1 Butterfly vertebra seen in patient 6, 7, 13 (arrows).

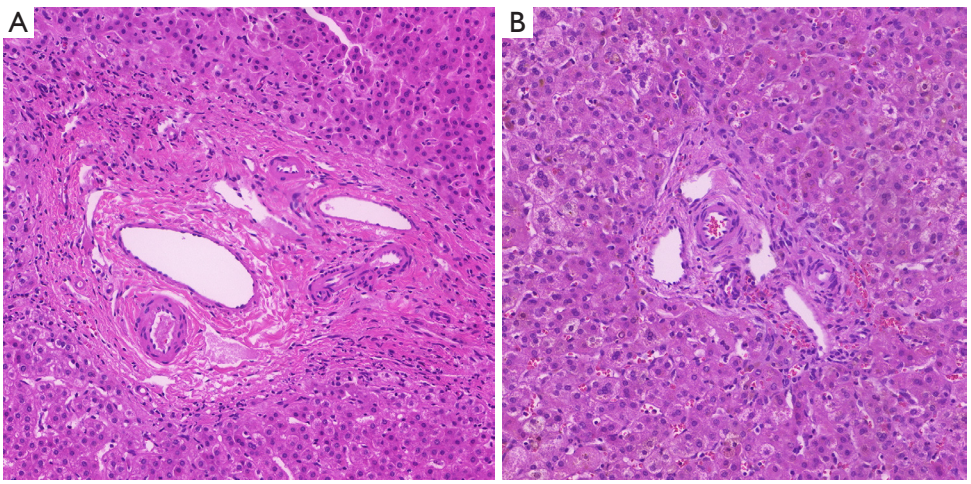

Figure 2 Liver biopsy histology in patient 5, 9 (HE staining, ×200). (A) No small bile duct was seen in the portal area. (B) Paucity of small bile ducts in the portal area, and some bile duct epithelium did not form a lumen. HE, hematoxylin and eosin.

Characteristic facial features were observed in 11 patients (78.6%). A total of eight patients (57.1%) had cardiac abnormalities, including two patients with ventricular septal defect with atrial septal defect, two patients with ventricular septal defect, two patients with atrial septal defect with patent ductus arteriosus, one patient with atrial septal defect and pulmonary artery stenosis, and one patient with pulmonary artery stenosis. In addition, one patient had renal dysplasia (polycystic kidney). There were six patients (42.9%) with vertebral abnormalities (*Figure 1*), including three patients with butterfly vertebra, one patient with butterfly vertebra and occult spina bifida, one patient with occult spina bifida, and one patient with irregular cone shape. Among the eight children who underwent ophthalmic examination, two (25.0%) had posterior embryotoxon. Liver biopsy was performed in eight children, among whom liver cell edema, cholestasis, and inflammatory cell infiltration were observed in the portal area (*Figure 2*). Of these eight patients, two were accompanied by small bile duct hyperplasia, two patients had no small bile ducts in the portal area, one patient showed the paucity of small bile ducts in the portal area and some bile duct epithelium did not form a lumen, and three patients had no bile duct hyperplasia or hypoplasia. In addition, five patients underwent cholangiography during laparoscopic exploration of the common bile duct (LECBD) and liver biopsy. The data of one patient was unknown. Among the four patients, normal-sized gallbladder was observed in one patient, and

Table 2 Biochemical results of 14 children with Alagille syndrome

Biochemical indexes	Minimum–Maximum	Reference range
Albumin (g/L)	37.8–50.1	32.0–52.0
Total bilirubin (μmol/L)	8.1–275.5	5.0–21.0
Conjugated bilirubin (μmol/L)	2.1–150.0	0–5.1
Unconjugated bilirubin (μmol/L)	2.3–124.9	1.0–20.0
Alanine transaminase (U/L)	43.0–477.0	<50.0
Aspartate transaminase (U/L)	65.0–370.0	15.0–60.0
γ-glutamyl transferase (U/L)	58.0–1,273.0	5.0–19.0
Alkaline phosphatase (U/L)	285.0–1,059.0	42.0–362.0
Serum bile acids (μmol/L)	35.2–368.7	0–12.0
Total cholesterol (mmol/L)	3.63–9.76	3.0–5.7
Triacylglycerol (mmol/L)	1.59–4.36	<1.7
Alpha-fetoprotein (ng/mL)		
<3 months	3,747.2–8,650	No range
3 to <6 months	19.17–30,629.82	5.15–274.7
6 to <12 months	No patient	2.66–148.21
≥12 months	0.87–3.27	0-20

small gallbladders were observed in three patients. The intrahepatic and extrahepatic bile ducts were visualized in two patients, the intrahepatic bile ducts were not visualized in two patients, and a narrow common hepatic duct was seen in one patient.

Biochemical results

In this study, 10 (71.4%) patients with ALGS had elevated total bilirubin and conjugated bilirubin to varying degrees, and nine of them were accompanied by elevated unconjugated bilirubin. Besides, 13 (92.9%) patients had elevated ALT, and all 14 patients (100%) presented with increased AST and γ-glutamyl transferase. Meanwhile, the serum bile acids and alkaline phosphatase levels were elevated in all the children (*Table 2*).

Gene variation

Genetic testing was performed for all 14 patients, including 12 patients (85.7%) with *JAG1* gene mutations and two patients (14.3%) with *NOTCH2* gene mutations. A total of 12 types of *JAG1* gene mutations were detected, including four nonsense mutations, three missense mutations, three frameshift mutations, and two splice mutations. There were two types of mutations in the *NOTCH2* gene, including one splice mutation and one frameshift mutation. Spontaneous mutations were seen in eight patients, three patients had mutations that originated from the father, and three patients had mutations that originated from the mother. In addition, six patients (46.2%) had new mutations (*Table 3*, *Figure 3*).

Follow-up

The duration of follow-up mean (Q1, Q3) was 48.5 (11.5, 69.0) months. Among the 14 children, one patient (7.1%) was small for gestational age at birth (SGA) and still had a delay in growth during the follow-up. Additionally, three patients (21.4%) experienced delayed growth between one and four years of age. There were two patients (14.3%) showing xanthomas at the ages of one and four years respectively. Persistent pruritus was observed in eight patients (57.1%). The liver function of one patient (7.1%) returned to normal after eight months of age. In addition, seven children (50.0%) had elevated bilirubin and 13 children (92.9%) had elevated ALT and/or AST. During

Table 3 Genetic test results of 14 children with Alagille syndrome

Patient No.	Mutant gene	Nucleotide changes	Amino acid changes	Mutation type	Exon	Family results	Literature report	ACMG pathogenicity analysis
1	*JAG1*	c.270delG	p.G90Gfs*71	Frameshift	Exon2	Spontaneous variant	Reported	Pathogenic (PVS1+PM2_Supporting+PS2)
2	*JAG1*	c.487C>A	p.P163T	Missense	Exon 4	Spontaneous variant	Reported	Pathogenic (PM2_Supporting+PP3_moderate+PS2+PS4_Supporting+PM5)
3	*JAG1*	c.694+1G>A	Splicing	Splicing	Exon 5	Mother	Reported	Likely pathogenic (PVS1_Strong+PM2_Supporting+PS4_Supporting)
4	*JAG1*	c.887-2A>G	Splicing	Splicing	Exon 8	Spontaneous variant	Reported	Likely pathogenic (PVS1_Moderate+PM2_Supporting+PS2+PS4_Supporting)
5	*JAG1*	c.2849_2850del	p.S950*	Nonsense	Exon 23	Spontaneous variant	Unreported	Pathogenic (PVS1+PM2_Supporting+PS2)
6	*JAG1*	c.550C>G	p.R184G	Missense	Exon 4	Mother	Reported	Pathogenic (PM2_Supporting+PP3_Strong+PM5+PM1+PP4)
7	*JAG1*	c.35_45delGCCCCCTAAGC	p.R12Pfs*57	Frameshift	Exon 1	Father	Unreported	Likely pathogenic (PM2_Supporting+PVS1)
8	*JAG1*	c.1156G>A	p.G386R	Missense	Exon 9	Spontaneous variant	Reported	Pathogenic (PS1+PS2+PM2+PP3)
9	*JAG1*	c.1860delC	p.F621Sfs*122	Frameshift	Exon 14	Spontaneous variant	Unreported	Pathogenic (PVS1+PS2+PM2)
10	*JAG1*	c.1293_1294insTAGTAGACA	p.A432*	Nonsense	Exon 10	Spontaneous variant	Unreported	Pathogenic (PVS1+PS2+PM2)
11	*NOTCH2*	c.6040_6041del	p.L2014Vfs*10	Frameshift	Exon 34	Mother	Unreported	Likely pathogenic (PVS1+PM2)
12	*NOTCH2*	c.1915+1G>T	Splicing	Splicing	Exon 11	Father	Unreported	Likely pathogenic (PVS1+PM2_Supporting)
13	*JAG1*	c.3140C>A	p.S1047*	Nonsense	Exon 25	Father	Reported	Pathogenic (PVS1+PM2+PP5)
14	*JAG1*	c.3006C>A	p.C1002*	Nonsense	Exon 24	Spontaneous variant	Reported	Pathogenic (PVS1+PM2_supporting+PS4_supporting+PS2_supporting)

ACMG, American College of Medical Genetics and Genomics.

the follow-up, two children stopped taking medication on their own, and 12 children took ursodeoxycholic acid and fat-soluble vitamins orally. Among these 12 children, six also had glycyrrhizin and/or glucuronolactone, four took cholestyramine, and one took rifampicin. No patients had hepatocellular carcinoma (HCC) during the follow-up.

Discussion

ALGS is an autosomal dominant disorder caused by mutations in the *JAG1* or *NOTCH2* gene. Its clinical manifestations are highly variable and involve multiple systems (24,25). In our study, all patients had liver involvement at the time of diagnosis, but their coagulation function was normal. We have summarized the data of Chinese children with ALGS in all the reported research articles (*Table 4*). Among these ALGS patients, 64.3% to 100% had cholestasis, 55.6–85.7% had cardiac abnormalities, 14.4–76.1% had vertebral abnormalities, 38.5–92.9% had characteristic facial features, 0–81.8% had posterior embryotoxon, and 5.6–68.8% had renal dysplasia. Consistent with previous studies, cholestasis was the most common clinical manifestation in the ALGS patients in our study. Nevertheless, in contrast to previous studies, the characteristic facial features were the second most frequently occurring symptom among the children with ALGS in the present study. A total of 78.6% of the ALGS children had characteristic facial features; the other three patients were too young, and no obvious facial features could be observed during their hospitalization. During the

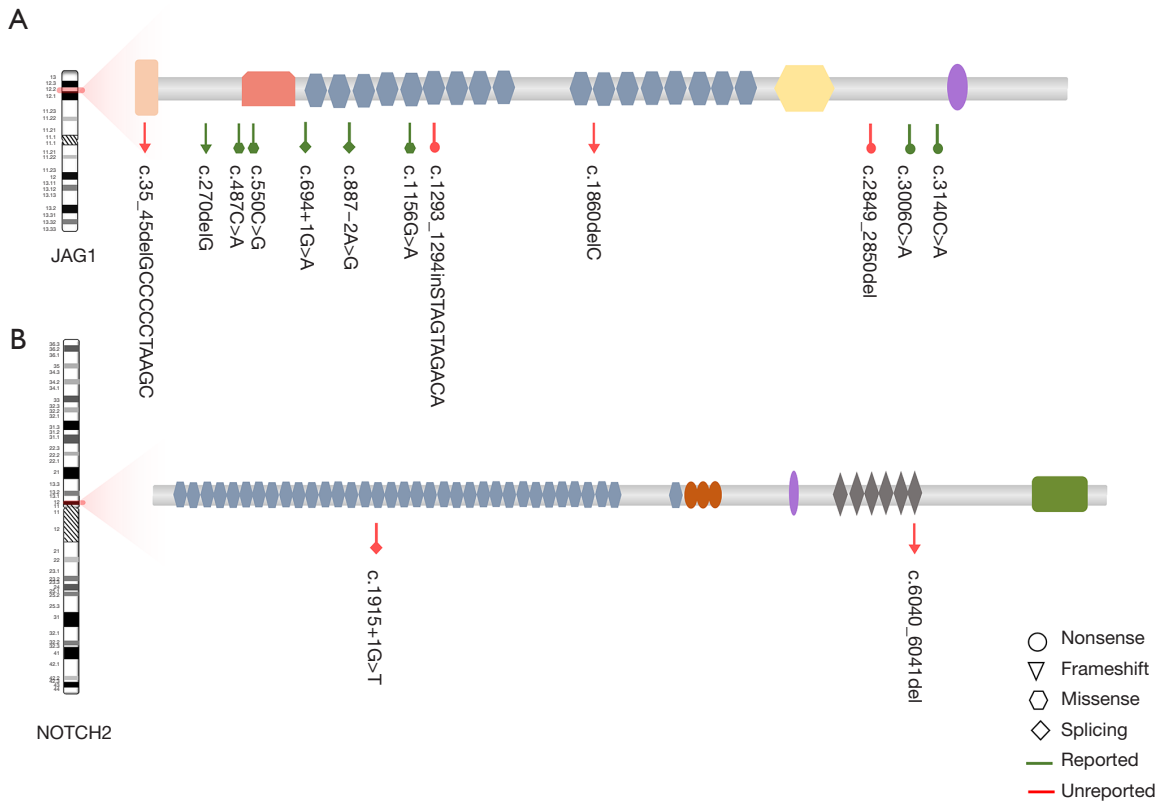

Figure 3 Schematic of JAG1 and NOTCH2 proteins with genetic variants in our study. (A) JAG1 and (B) NOTCH2 proteins are depicted with all detected variants shown below the schematic. Numbers indicate amino acid coordinates. Protein domains include (JAG1): signal peptide (orange), DSL domain (red), EGF-like repeats (blue), cysteine-rich domain (yellow), and transmembrane domain (purple); and (NOTCH2): EGF-like repeats (blue), LNR domain (brown), transmembrane domain (purple), ANK repeats (grey), PEST domain (green). DSL, Delta Serrate LAG2; LNR, Lin12/Notch repeats; ANK, ankyrin; PEST, proline, glutamate, serine, threonine-rich.

follow-up, two patients did not have special facial features, and the other patient was followed up for a short time and needs to be further determined in the future. Cardiac abnormalities were the second most common manifestation among the Chinese children with ALGS in previous data. In addition, 27.8–54.5% of the ALGS children showed pulmonary artery stenosis (15-21). However, only two patients (14.3%) had pulmonary artery stenosis, and the detection rate was lower than that reported in previous studies on Chinese children with ALGS. The incidence of vertebral abnormalities (42.9%), posterior embryotoxon (25.0%), and renal abnormalities (7.1%) was lower than the overall rate reported previously, possibly due to the small sample size or ethnic origin.

The typical pathological manifestation of ALGS is the paucity of intrahepatic bile ducts. Considering that liver biopsy is an invasive examination, only eight children underwent liver biopsy in this study. Among these patients, seven had the liver biopsy within one year of age, six within six months of age, and one at more than five years of age. The liver pathology of eight children showed hepatocellular edema, cholestasis, and infiltration of inflammatory cells in the portal area. Among them, two patients were accompanied by small bile duct hyperplasia, two patients had no small bile ducts in the portal area, one patient showed the paucity of small bile ducts in the portal area and some bile duct epithelium did not form a lumen, and three patients had no bile duct hyperplasia or hypoplasia. Previous studies showed that bile duct deficiency was more common in older children than in infants younger than six months in the liver biopsy pathology of ALGS patients (4,26). In this study, bile duct deficiency accounted for 33.3% of the children younger than six months on liver biopsy, and 50.0% of children older than six months on liver biopsy, which are identical with those reported in literature. However, the detection rate of bile duct deficiency was

Table 4 Summary of the data of Chinese children with ALGS in all the reported research articles

Research article	Cholestasis	Cardiac abnormalities	Vertebral abnormalities	Characteristic face	Posterior embryotoxon	Renal dysplasia	Interlobular bile duct paucity	Gene detection
Li L *et al.* (14)	90/91	78/91	67/88	66/90	30/69	12/66	21/31	*JAG1* gene mutations in 70 patients, no mutations in 21 patients
Guo L *et al.* (15)	11/11	8/11	7/11	9/11	9/11	2/11	NA	*JAG1* gene mutations in 11 patients
Liu Y *et al.* (16)	15/16	12/16	7/16	7/16	NA	11/16	4/8	*JAG1* gene mutations in 15 patients, *NOTCH2* gene mutation in 1 patient
Chen YX *et al.* (17)	12/13	8/13	1/7	5/13	1/7	NA	1/9	*JAG1* gene mutations in 8 patients, *NOTCH2* gene mutations in 4 patients
Liu XG *et al.* (18)	18/18	10/18	11/18	15/18	5/16	1/18	NA	*JAG1* gene mutations in 13 patients, *NOTCH2* gene mutations in 3 patients, *JAG1* gene mutations + *NOTCH2* gene mutations in 2 patients
Chen Y *et al.* (19)	8/10	6/10	4/10	7/10	0/10	3/10	1/2	*JAG1* gene mutations in 9 patients, *NOTCH2* gene mutation in 1 patient
Li D *et al.* (20)	9/14	10/14	2/13	13/14	1/13	NA	2/6	*JAG1* gene mutations in 7 patients, *NOTCH2* gene mutations in 2 patients, no mutations in 3 patients
Yan J *et al.* (21)	16/17	12/16	12/17	15/17	7/12	NA	12/15	*JAG1* gene mutations in 13 patients, no mutations in 2 patients

Data are presented as number of positive/total. NA, not available; ALGS, Alagille syndrome.

lower than that reported in previous studies, which may be related to the small sample size, young biopsy age, or mild comparison of symptoms in children in this study. In addition, there were two cases (25%) of children with liver pathology indicating small bile duct hyperplasia (the biopsy ages were six months and two months). This phenomenon did not only occur in this study (4,19), suggesting that bile duct hyperplasia may exist in some children with ALGS, and it is necessary to distinguish it from BA to avoid misdiagnosis.

ALGS is caused by *JAG1* and *NOTCH2* gene mutations in the NOTCH signaling pathway. The protein encoded by the *JAG1* gene acts as a Delta ligand for the Notch receptor. The protein encoded by the *NOTCH2* gene is a receptor that enables molecular signaling between cells. Mutations in these genes contribute to various systemic disorders, possibly on account of the loss of cellular interactions (27). Other genes function as genetic modifiers, such as *THBS2*, *POGLUT1*, and members of the Fringe protein family, such as *LFNG*, *RFNG*, and *MFNG* (28,29). These proteins have the ability to modify the NOTCH signaling pathway. It is reported that 94.3% of clinically diagnosed ALGS patients can be attributed to the variants in the *JAG1* gene, around 2.5% of ALGS patients are caused by pathogenic variants in the *NOTCH2* gene, and approximately 3.2% of ALGS patients with unknown pathogenic genes (13). In the previous reported research articles of Chinese ALGS patients, 146 patients (78.9%) had *JAG1* gene mutations, 11 patients (5.9%) had *NOTCH2* gene mutations, two patients (1.1%) had both *JAG1* gene mutations and *NOTCH2* gene mutations, and 26 patients (14.1%) had none of the aforementioned gene variants (*Table 4*). A total of approximately 700 types of *JAG1* variants and 44 types of *NOTCH2* variants in humans have been found in recent literature reports. Among them, *JAG1* mutations are primarily protein-truncating variants, and *NOTCH2* mutations are primarily missense mutations (13,22,30). In our study, the mutation profiles of the *JAG1* and *NOTCH2* genes were very dispersed. A total of 12 different *JAG1* gene mutations and two different *NOTCH2* gene variations were detected. In contrast, the *NOTCH2* gene mutations identified in this study were novel splicing (c.1915+1G>T) and frameshift (c.6040_6041del) mutations. Cardiac involvement, vertebral anomalies, and facial features are less

frequent in ALGS patients with *NOTCH2* mutations (31). In our study, vertebral abnormalities, posterior embryotoxon, and renal dysplasia were not seen in *NOTCH2* mutation patients. This situation might be attributed to the limited number of patients involved. With the aim of obtaining a more in-depth understanding of the relationship between the genotypes and phenotypes of Chinese ALGS children, we conducted a summary of the children with detailed information from previous research articles (14-16,18,19,21) (Table S1). After combining the patients from previous studies with those in our study, we found that none of the four children with *NOTCH2* mutations who had undergone ophthalmological examination had posterior embryotoxon. There was a trend that cardiac abnormalities, vertebral abnormalities, characteristic facial features, posterior embryotoxon, and renal dysplasia occurred more frequently in ALGS patients with *JAG1* mutations. In addition, there were no hotspot mutations in the *JAG1* or *NOTCH2* genes in Chinese ALGS patients. The most common type of *JAG1* mutation was c.2698C>T (six patients), followed by c.439+1G>A (four patients). Among the six patients with the *JAG1* mutation c.2698C>T and four patients with the *JAG1* mutation c.439+1G>A, diverse clinical manifestations were observed in ALGS patients with the same gene mutations. This finding indicated that there were no genotype-phenotype correlations among the ALGS patients, which was in accordance with previous studies (3,32).

There were still limitations in our study. The samples in this study were all from Zhejiang. This geographical limitation may make the research results more one-sided. In the future, we need to expand the sample size and conduct multicenter research to obtain a more comprehensive and extensive analysis of ALGS.

In general, the clinical manifestations of ALGS are diverse, and early diagnosis of atypical children is difficult and prone to misdiagnosis as BA. Gene testing provides effective assistance for the early diagnosis of this disease. In our study, 46.2% of the children detected new mutations, which enriched the genetic spectrum of mutations in ALGS in China. Moreover, we summarized the data of Chinese ALGS patients in all reported research articles, thus proving a more comprehensive understanding of the clinical and genetic characteristics of ALGS patients in China.

Conclusions

ALGS presents a variety of clinical manifestations. Some patients may be misdiagnosed with BA in the early stage. Genetic testing is helpful for early diagnosis. Maralixibat, a new drug, reduces bile acid enterohepatic circulation and treats cholestasis in ALGS patients, leading to an earlier improvement in quality of life (33,34). The spectra of the *JAG1* and *NOTCH2* genes are abundant and many novel mutations have been identified in Chinese children with ALGS. Here, we have reported six new mutations in both *JAG1* and *NOTCH2*. In summary, this study presents new gene mutations and enriches the present data concerning ALGS patients in China.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Medical Ethics Committee of Children's Hospital, Zhejiang University School of Medicine (No. 2023-IRB-0168-P-01) and individual consent for this retrospective analysis was waived.

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