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Clinical, pathological and genetic characteristics of 17 unrelated children with Alagille Syndrome

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Abstract

Background Alagille syndrome (ALGS) is a multisystem genetic disorder frequently characterized by hepatic manifestations. This study analyzed the clinical, pathological, and molecular genetic features of ALGS to improve the efficiency of clinical diagnosis.

Methods We retrospectively analyzed the clinical manifestations, pathological examination findings, and genetic testing results of 17 children diagnosed with ALGS based on the revised criteria and hospitalized at our center from January 2012 to January 2022.

Results The clinical manifestations are as follows: Cholestasis (16/17, 94%), characteristic facies (15/17, 88%), heart disease (12/16, 75%), butterfly vertebrae (12/17, 71%) and posterior embryotoxon (7/12, 58%). Among the 15 patients who underwent liver pathology examination, 13 (87%) were found to have varying degrees of bile duct paucity. Genetic testing was performed on 15 children, and pathogenic variants of the jagged canonical Notch ligand 1 (*JAG1*) gene were identified in 13 individuals, including 4 novel variants. No pathogenic variant in the notch homolog 2 (*NOTCH2*) gene were identified, and 2 children exhibited none of the aforementioned gene pathogenic variants. The median follow-up duration was 7 years. Of the remaining 15 patients (excluding 2 lost to follow-up), 11 remained stable, 4 deteriorated, and no patient died during the follow-up period.

Conclusions Among children diagnosed with ALGS, cholestasis stands as the most common feature. To minimize the risk of misdiagnosis, genetic testing should be performed on children exhibiting cholestasis, followed by the application of the revised diagnostic criteria for ALGS. While pharmacological therapy has shown effectiveness for ALGS patients, liver transplantation may be considered in instances of severe pruritus.

Keywords Alagille syndrome, Clinical manifestation, Cholestasis, Genetic analysis

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Introduction

Alagille Syndrome (ALGS) is a rare multisystem disorder with an estimated incidence rate ranging from 1 in 30,000 to 1 in 100,000 individuals [1]. It is inherited in an autosomal dominant manner in approximately 40% of patients [2]. ALGS is primarily attributed to pathogenic variants in the jagged canonical Notch ligand 1 (*JAG1*) and Notch Homolog 2 (*NOTCH2*) genes, which encode molecules involved in the NOTCH signaling pathway [3–5]. The severity of ALGS can range from asymptomatic presentations to fatal complications, with a mortality rate reaching up to 10% [4, 6, 7]. The clinical manifestations of ALGS are diverse, including cholestasis, characteristic facies, posterior embryotoxon, abnormal development of the heart and bones, kidney and vascular abnormalities in some patients, and liver histopathology may reveal the absence of intrahepatic bile ducts [2]. However, due to the variable expressivity of clinical manifestations among ALGS patients, some exhibit only a single typical symptom, and some are even in a subclinical state when seeking medical attention [8–12]. Consequently, relying on classical diagnostic criteria may lead to overlooking the diagnosis of ALGS [13, 14]. The current diagnosis primarily relies on the revised diagnostic criteria introduced in 2007 [15, 16], which incorporate genetic testing to improve diagnosis accuracy. We retrospectively analyzed the clinical data of 17 children diagnosed with ALGS at our hospital over the past 10 years, aiming to provide compelling evidence supporting the early detection of ALGS.

Materials and methods

Patients

The study enrolled 17 children diagnosed with ALGS who were admitted to the Fifth Medical Center of the PLA General Hospital from January 2012 to January 2022. ALGS diagnosis was established based on the revised criteria [15, 16], excluding children with comorbidities. Cholestasis was defined by elevated serum direct/conjugated bilirubin levels (>1.0 mg/dL or >17μmol/L), or by gamma-glutamyl transpeptidase (GGT) levels surpassing 3 times the upper limit of normal [17, 18]. Characteristic facies is described as an inverted triangular face, defined by a prominent forehead, deep-set eyes with hypertelorism, a straight nose with a bulbous tip, and a pointed chin [2].

Clinical data were gathered from medical records and follow-up visits. At diagnosis, demographic characteristics and clinical data were recorded, including clinical symptoms, laboratory data (liver function and lipid tests), physical examination results (growth and development condition, facial features, and indications associated with chronic hepatitis, such as jaundice, spider nevi, or palmar erythema), routine examinations (cardiac and abdominal ultrasound, chest and abdominal Computed Tomography, abdominal Magnetic Resonance Imaging, and spinal X-ray), and specialist examinations (ophthalmic slit-lamp examination). Additionally, results from liver biopsy and genetic testing were obtained. The study was ethically approved by the Ethics Committee at the Fifth Medical Center of the PLA General Hospital. All clinical information and images presented in this report were acquired with prior written consent from the patients' parents.

Percutaneous liver pathology

Percutaneous liver biopsies were performed on 15 cases. The paraffin-embedded tissue was sectioned and stained with hematoxylin, eosin, Masson's trichrome, cytokeratin-7 and cytokeratin-19. The histological examination was reviewed by experienced pathologists. Bile duct paucity was defined as the absence of interlobular bile ducts in at least 50% of the portal tracts within liver samples containing 10 or more portal areas [19].

Genetic variation analysis

DNA was extracted from peripheral blood, with detailed methodology outlined in the previous publications [20, 21]. Briefly, the sequenced data were then aligned with the human reference genome version 19 (hg19) using the Burrows-Wheeler Alignment (BWA) Tool (http://bio-bwa.sourceforge.net/). The Genome Analysis Toolkit (GATK) software (www.broadinstitute.org/gatk) was used to analyze single-nucleotide polymorphisms (SNPs). ANNOtation VARiants (ANNOVAR) was utilized for annotating candidate variants(annovar.openbioinformatics.org/en/latest/). MutationTaster [22], Sorting Intolerant From Tolerant (SIFT) [23, 24] and Polyphen-2 [25] programs were used to predict the potential impacts of single-nucleotide variants (SNVs). Following the American College of Medical Genetics and Genomics (ACMG) guideline [26], pathogenic variants were classified as benign, likely benign, variants of unknown clinical significance (VUS), likely pathogenic and pathogenic. The candidate causal variants identified via whole-exome sequencing (WES) were subsequently confirmed using Sanger sequencing. Co-segregation analyses were also performed within the patient's family. Fragments covering mutated sites were amplified, purified with the Zymoclean Polymerase Chain Reaction (PCR) Purification Kit (Zymo Research, USA), and sequenced using an ABI 3730 DNA Sequencer (SeqGen, CA). Sanger sequencing data were analyzed by Chromas Lite v2.01 (Technelysium Pty Ltd., Tewantin, QLD, Australia).

Treatment and follow-up

Except for 2 patients who opted out of medication owing to their mild symptoms, the other 15 patients underwent prolonged oral therapy, which comprised

of ursodeoxycholic acid, cholestyramine, bicyclic alcohol, and compound glycyrrhizin tablets, as well as various vitamin supplements. Children exhibiting severe cholestasis or pruritus underwent liver transplantation. Every three months, the children's liver function, biochemistry, blood routine, and abdominal ultrasound were reviewed, along with regular assessments of their growth and development. Treatment adjustments were made in accordance with changes in the patients' conditions. All patients were followed up until August 2023.

Statistical analysis

Continuous variables were shown as medians and interquartile ranges. Categorical variables were presented as counts and percentages. Statistical analysis was performed with R Foundation for Statistical Computing software (v. 4.1.3; Vienna, Austria; http://www.r-project.org/).

Results

Clinical characteristics

This study included a total of 17 participants, with 10 males and 7 females. The median age of onset among the 17 children was 2 months (range: 1-168 months), with most developing the disease within 3 months of birth. The median age at diagnosis was 5 years (range: 1–15 years).

All patients in the study exhibited liver involvement. Cholestasis was the most prevalent characteristic, affecting 94% of the patients (16/17). Characteristic facies was the second most prevalent, presenting in 88% of patients (15/17) (Fig. 1). Heart disease was identified in 75% of patients (12/16), and butterfly vertebrae were detected in 71% (12/17) (Fig. 2). The posterior embryotoxon was detected in approximately 58% of patients (7/12) assessed. Growth retardation was observed in 53% of the children (9/17), and pruritus was reported by 42% (7/17). Only Patient 8 had a relevant family history, specifically a history of ALGS in an elder brother. Abdominal MRI scans revealed cirrhosis with ascites in patients 5 and 11, which was histologically confirmed. Both patients, aged over 10 years, exhibited significant cholestasis upon admission. Detailed clinical information for each patient is provided in Table 1 and Supplementary Table 1.

Biochemical results

Laboratory examination indicators for all patients are presented in Table 2. AST, GGT, and LAP levels were elevated in all patients. Total biliary acid (TBA) was increased in 16 patients (94%), LAP in fifteen patients (88%), alanine aminotransferase (ALT) in thirteen patients (76%), total cholesterol (TC) in thirteen patients (76%), triglycerides (TG) in twelve patients (71%), DBil in seven patients (42%), and alkaline phosphatase (ALP) levels in five patients (29%).

Histological results

15 children underwent liver biopsy for pathology examination, revealing intrahepatic bile duct paucity in 12 cases (Fig. 3). Mild liver inflammation and fibrosis were

Fig. 1 Characteristic facies of Alagille syndrome in case 2: a child aged 1 year and 7 months with a prominent forehead, deep-set eyes with hypertelorism, a straight nose with a bulbous tip, and a pointed chin

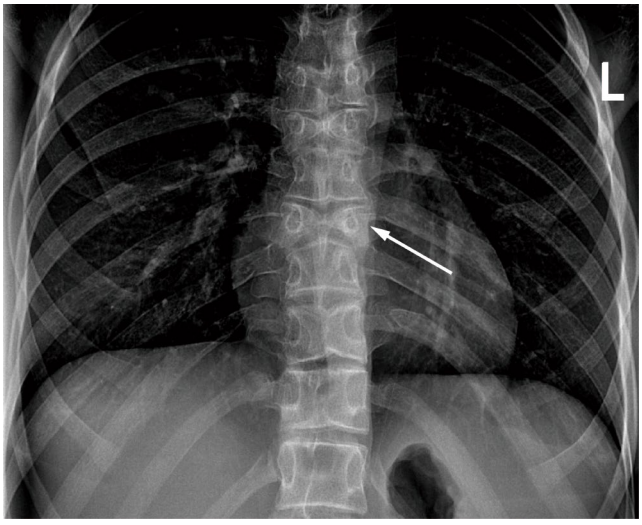

Fig. 2 Butterfly vertebrae in case 11 (The lesions occurred in the thoracic vertebra region, as indicated by an arrow)

both present in 14 patients. The liver biopsy of one patient indicated cirrhosis (Table 3 and supplementary Table 2).

Genetic test results

Among the 15 individuals subjected to genetic testing, 13 carried pathogenic variants in the *JAG1* gene, while none exhibited pathogenic variants in the *NOTCH2* gene. No *JAG1* gene pathogenic variants were detected in two patients. Thirteen distinct pathogenic variants in the *JAG1* gene were identified in the 13 children harboring such variants, comprising six frameshift, three nonsense, two missense, one splice-site mutation, and one large fragment heterozygosity loss. Four novel pathogenic variants in the *JAG1* gene were identified in our study. All pathogenic variants identified in our study were either previously reported or predicted to be pathogenic. The amino acid changes induced by these pathogenic variants are located at diverse positions on the *JAG1* protein. Bioinformatics software was used to assess the pathogenicity of an unreported missense mutation, c.548T>A (p.I183N), which was deemed harmful by MutationTaster, SIFT, and Polyphen-2 (Table 4, Fig. 4 and Fig. S1).

Management and follow-up

After a period of treatment, most children demonstrated improved liver function test results, yet their pruritus did not experience significant relief. Liver transplantation was performed for case 8 (presenting with severe pruritus and stunted growth) and case 11 (suffering from severe cholestasis).

All patients underwent follow-up for a median duration of 7 years. During this period, 2 patients were lost to follow-up, 11 remained stable, and 4 experienced deteriorated. None of the children in the study developed liver cancer or succumbed to the disease. Apart from cases 5 and 11, who were initially diagnosed with decompensated cirrhosis, no other child progressed to this condition. Cases 8 and 11, who underwent liver transplantation, were monitored for 2 years post-surgery. Presently, both are administered tacrolimus orally as an anti-rejection measure. Although their symptoms of jaundice and pruritus resolved post-transplantation, growth and development of case 8 remained below normal levels, while case 11 exhibited postoperative anemia.

Discussion

ALGS, a multisystem disorder affecting multiple organs, was first reported by Alagille in 1969, with classical diagnostic criteria were established in 1975 [27]. The classical diagnostic criteria require histological confirmation of bile duct paucity and with a minimum of three additional characteristic symptoms [13, 14, 27]. Due to the stringency of the classical diagnostic criteria, only 12 children in our study met the criteria. Currently, the diagnosis of ALGS predominantly relies on revised criteria [15, 16] that emphasize the diagnostic significance of *JAG1* gene pathogenic variants, enhancing diagnostic efficiency.

Cholestasis is widely regarded as a characteristic symptom in children with ALGS [2, 28–30]. In this study, 94% of patients presented with cholestasis during consultation. This percentage is comparable to the 89% incidence of cholestasis reported in the King's College case series [31]. Bile duct paucity or hypoplasia is a key symptom of ALGS. Among the 15 patients who underwent liver pathology examination in this study, 13 (87%) patients had bile duct defects of varying degrees, a higher proportion than the 75% incidence of bile duct paucity reported by King's College [31]. However, other studies indicate that the incidence of bile duct paucity in children with

Table 1 Clinical characteristics of 17 children with ALGS

Patient	Sex	Age of onset	Diagnostic age	Family history	Pruritus	Cholestasis	Posterior embryotoxon	Characteristic facies	Butterfly vertebra	Cardiac abnormality	Reason for medical treatment	Last follow-up age
1	Male	< 1 month	4 years	-	-	+	+	+	-	+[a]	jaundice; cardiac abnormality	16 years
2	Male	< 1 month	3 years	-	+	+	-	+	-	+[b]	pruritus; jaundice; cardiac abnormality	12 years
3	Male	1 month	10 years	-	-	+	-	+	+[h]	+[c]	jaundice; cardiac abnormality	18 years
4	Male	3 months	4 years	-	-	+	+	+	+[i]	+[d]	jaundice; cardiac abnormality	12 years
5	Male	120 months	12 years	-	-	+	-	+	+[j]	+[e]	jaundice; cardiac abnormality	19 years
6	Female	24 months	3 years	-	-	+	N/A	-	+[g]	N/A	jaundice	8 years
7	Female	< 1 month	10 years	-	-	+	N/A	-	+[k]	+[c]	jaundice; cardiac abnormality	16 years
8	Female	< 1 month	1 year	+	+	+	-	+	+[l]	+[b]	pruritus; jaundice; cardiac abnormality	6 years
9	Female	3 months	4 years	-	+	+	+	+	+[m]	+[f]	pruritus; jaundice; cardiac abnormality	9 years
10	Male	36 months	3 years	-	-	+	N/A	+	+[k]	-	jaundice	9 years
11	Female	168 months	15 years	-	-	+	+	+	+[n]	+[g]	jaundice; cardiac abnormality	21 years
12	Male	< 1 month	5 years	-	+	+	+	+	-	+[c]	pruritus; jaundice; cardiac abnormality	13 years
13	Male	36 months	5 years	-	-	-	N/A	+	+[o]	+[c]	cardiac abnormality	6 years
14	Male	< 1 month	8 years	-	+	+	+	+	-	-	pruritus; jaundice	15 years
15	Male	2 months	6 years	-	+	+	+	+	+[j]	-	pruritus; jaundice	18 years
16	Female	36 months	6 years	-	+	+	N/A	+	-	+[b]	pruritus; jaundice; cardiac abnormality	15 years
17	Female	3 months	4 years	-	-	+	-	+	+[n]	-	jaundice	9 years

(a) hypoplasia of the left pulmonary artery; (b) pulmonary arterial stenosis accompanied by atrial septal defect or patent foramen ovale; (c) mild regurgitation of the tricuspid valve; (d) perimembranous ventricular septal defects and pulmonary arterial stenosis; (e) mild regurgitation of the mitral valve; (f) patent ductus arteriosus, atrial septal defect, mild regurgitation of the tricuspid valve, and venous sinus dilation; (g) dilation of the coronary sinus; (h) at thoracic vertebra 5; (i) at thoracic vertebra 3–8; (j) incomplete butterfly vertebra; k) at thoracic vertebra 6; l) at thoracic vertebra 4, 5, 7 and 10; m) at thoracic vertebra 9; n) at thoracic vertebra 5, 6 and 8; o) at lumbar vertebra 5 and sacral vertebra; n) at thoracic vertebra 6–7

ALGS can reach up to 85% [32]. Overall, bile duct paucity is the most consistent feature of ALGS [33], and different detection rates may be related to subject age and errors in liver biopsy. To differentiate biliary atresia (BA), biliary exploration was performed on two children (cases 3 and 7) at 2 months of age. There have been reported cases where ALGS was erroneously diagnosed as biliary atresia, ultimately leading to death or liver transplantation following Kasai surgery [34, 35]. Hence, in cases of suspected biliary atresia, biliary exploration is crucial to exclude ALGS.

Characteristic facies is a well-known prominent feature of patients with ALGS, particularly those harboring pathogenic variants of the *JAG1* gene [36, 37]. Characteristic facies can be identified in 80% of children with ALGS [38], while in this study, 88% of the patients exhibited this typical feature. Prompt identification of suspicious facial features in children is crucial. However, characteristic facies may not be apparent in early infancy [39, 40]. Furthermore, the use of characteristic facies in diagnosis remains controversial due to the subjectivity of facial observation and variations in observer interpretation [39]. Heart disease is the most common extrahepatic manifestation of ALGS, with an overall incidence of 94% [33]. Among these, the pulmonary artery is the most common site of anomalies, accounting for approximately 76% of all patients with cardiac anomalies [41]. In this study, approximately 75% of patients exhibited cardiac anomalies, whereas only 25% presented pulmonary artery anomalies. Although complex cardiac lesions are recognized as a significant cause of mortality in children with ALGS [32], none of the children in our follow-up study succumbed. The prevalence of butterfly vertebrae in our study was 71%, aligning with previous reports that ranged from 33–87% [42]. Most skeletal abnormalities in the studied children were predominantly found in the thoracic vertebrae, with only case 13 showing lumbar and sacral vertebrae affected. Posterior embryotoxon is the most common ocular abnormality in patients with ALGS, which usually does not affect vision and occurs in 56-95% of ALGS patients [43, 44]. Among the 12 children who underwent ophthalmic slit lamp examination in this study, 7 were found to have posterior embryotoxon, representing approximately 58% of the examined population.

Genetic testing in this study revealed a pathogenic variant detection rate of 87% for the *JAG1* gene, whereas no pathogenic variant was identified in the *NOTCH2* gene. These findings are consistent with the conclusions of previous studies [12]. As an important ligand of the Notch signaling pathway, changes in the structure and function of *JAG1* protein significantly affect Notch signaling levels [12]. The NOTCH signaling pathway has been found to be involved in the development of multiple systems, often manifesting as multi-system symptoms in patients with ALGS [45]. However, genetic testing in this study yielded negative results for two children. This phenomenon has also been reported in previous studies [27, 46, 47], we speculate that it may be related to the fact that whole-exome sequencing technology is prone to miss deletions. Additionally, we noticed that four patients inherited their *JAG1* gene pathogenic variants from their "healthy" mothers. These mothers exhibited no overt clinical symptoms during their visits, and due to limitations, liver pathology examinations were not conducted on these apparently "healthy" mothers. We speculate that the asymptomatic carriage of *JAG1* gene pathogenic variants may be due to differential expression of the *JAG1* gene in hepatoblasts and portal vein mesenchyme (PVM). Previous studies have suggested that the absence of *Jag1* in hepatoblasts does not affect bile duct development in mice. In addition, the correlation between ALGS genotype and phenotype remains unclear, and some modifier genes are thought to impact the phenotype of patients with ALGS [48].

Ursodeoxycholic acid is currently the first-line therapy for ALGS and has been proven effective in controlling patients' pruritus and xanthoma formation [49]. Although ursodeoxycholic acid treatment yielded positive therapeutic outcomes for these children, a subset experienced minimal relief from pruritus. To address this issue, we added choline amine as adjuvant therapy, which effectively controlled the children's pruritus. Clinically, however, liver transplantation remains necessary for 21-31% of patients due to severe pruritus [50], with our study observing a 29% rate. While liver transplantation significantly improves patients' original symptoms, postoperative complications and the long-term need for immunosuppressants significantly affect their quality of life, echoing findings from previous studies [51, 52]. Due to advancements in treatment modalities and improved survival rates following liver transplantation (LT), over 90% of children with ALGS now survive into adulthood [2, 29]. By the end of the follow-up, all study patients had reached adulthood with stable disease conditions. Hence, it is imperative for adult hepatologists to possess a thorough understanding of the clinical manifestations and therapeutic approaches associated with ALGS. Such knowledge is essential to address the evolving healthcare demands of ALGS patients transitioning from pediatric to adult medical care [2].

This study had several limitations. Firstly, as a retrospective, single-center study, it was constrained by a limited sample size. Secondly, the study's subjects were exclusively hospitalized patients with relatively severe conditions, potentially introducing bias. In future studies, we plan to expand the sample size and include more outpatient cases. Lastly, all children in this study underwent whole exome sequencing for genetic testing. The

Table 2 Biochemical characteristics of 17 children with ALGS

Patient	TBil (μmol/L)	DBil (μmol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TBA (μmol/L)	LAP (U/L)	TC (mmol/L)	TG (mmol/L)	CHE (U/L)	ALB (g/L)
1	16.7	5.3	166	122	555	266	84	425	11.25	1.66	6751	43
2	12.6	7.1	203	169	922	701	41	800	10.23	1.84	6336	42
3	26.9	16	71	228	398	381	32	388	7.84	0.81	6055	45
4	15.8	8.2	18	55	443	374	113.9	370	7.88	2.32	5377	37
5	264.5	219.8	171	383	184	97	373	64	3.21	1.43	1609	38
6	9.1	4.5	136	116	650	445	18	575	8.65	0.49	6507	43
7	92.4	70.7	236	184	222	201	214	122	4.09	1.95	2157	29
8	23.8	20	99	102	501	198	139	417	7.07	2.31	4479	37
9	16.7	9.5	18	47	581	228	88.9	406	7.7	1.62	5760	41
10	8.9	2.8	67	57	318	213	26	74	2.43	0.88	8653	45
11	79	57	117	154	154	79	126	117	6.5	1.14	3670	41
12	28.9	23.5	329	118	728	514	108	476	11.6	1.21	4548	35
13	8.6	2.9	69	61	244	74	7	115	4.26	0.73	7462	41
14	34.2	23.5	29	152	650	453	48	288	10.44	1.74	6387	39
15	12.2	4.4	130	116	667	433	41	443	7.26	1.13	5595	39
16	16.4	8	277	158	592	438	21	579	9.69	1.13	8233	42
17	6.2	8.68	19	122	535	475	58	402	8.68	1.55	6520	38

TBil, total bilirubin; DBil, direct bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; TBA, total bile acid; LAP, leucine arylamidase; TC, total cholesterol; TG, triglyceride; CHE, cholinesterase; ALB, albumin; ↑, elevated; ,↓,decreased

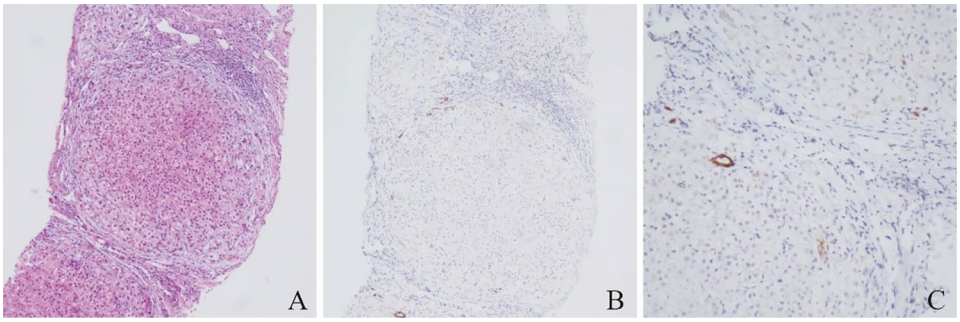

Fig. 3 The histopathological change of liver biopsy in case 11. **A**: Liver cell swelling, fibrous tissue proliferation and few bile duct in the portal area (HE, original magnifi cation × 100); **B**: Few bile duct in the portal area (CK-7/19, original magnifi cation × 100); **C**: Few bile duct in the portal area (CK-7/19, original magnifi cation × 200)

Table 3 Pathological results of liver puncture in 15 children[a] with ALGS

Patient	Paucity of intrahepatic bile duct	Enlarged portal area	Inflammation grade	Fibrosis stage
1	+	+	G1	S1-2
2	+	-	G0-1	S1
3	-	+	G1	S1
4	+	+	G0-1	S1-2
6	+	-	G1	S0
7	-	-	G1	S0
8	+	-	G0-1	S0
10	-	-	G0-1	S0
11	+	+	G2	S4
12	+	+	G0-1	S1
13	+	-	G0-1	S0
14	+	+	G0-1	S0
15	+	+	G1	S2
16	+	+	G0-1	S0
17	+	-	G1	S0

[a] 2 patients did not receive liver biopsy

limitations of this sequencing technology may result in missed detections of deletions. Furthermore, it is crucial to acknowledge the challenges posed by the homology in covering exons 1–4 of the *NOTCH2* gene, which may potentially lead to their omission during exome sequencing.

Conclusion

Cholestasis is the most prevalent characteristic observed in children diagnosed with ALGS. Genetic testing should be conducted for children presenting with cholestasis, followed by the application of revised diagnostic criteria for ALGS to minimize misdiagnosis. Pharmacological therapy has proven effective for patients with ALGS, while liver transplantation may be considered in cases of severe pruritus.

Table 4 Genetic traits of children with Alagille syndrome

Patient	cDNA Variant	Amino acid change	Exon/Intron	Mutation type	Protein Region	Parental Origin	Classification according to ACMG [26, 53]	Reports
1	c.232_233dup	p.L79Afs*83	Exon2	Frameshift	MNNL	Unknown	Pathogenic (PVS1 + PM2_Supporting + PP4)	This study
2	c.439 + 1G > A	?	Intron3	Canonical-splice	-	Unknown	Pathogenic (PVS1 + PS4 + PM2_Supporting)	[3, 54–58]
3	c.2323G > T	p.E775*	Exon18	Nonsense	EGF	Unknown	Pathogenic (PVS1 + PS4_Supporting + PM2_Supporting)	[59]
4	c.3000del	p.I1000Mfs*36	Exon24	Frameshift	-	*De novo*	Pathogenic (PVS1 + PM2_Supporting + PP4)	This study
5	c.550 C > T	p.R184C	Exon4	Missense	DSL	*De novo*	Pathogenic (PP3_Strong + PM2_Supporting + PS2_VeryStrong)	[60, 61]
6	c.1308 C > A	p.C436*	Exon10	Nonsense	EGF_CA	Mother	Pathogenic (PVS1 + PM2_Supporting + PS4_Supporting)	[62]
7	c.3002_3003del	p.A1001Vfs*10	Exon24	Frameshift	-	*De novo*	Pathogenic (PVS1 + PM2_Supporting + PS4_Supporting)	[61]
8	c.548T > A	p.I183N	Exon4	Missense	DSL	Mother	Likely pathogenic (PP3_Strong + PM2_Supporting + PP2 + PP4)	This study
9	c.2122_2125del	p.Q708Vfs*34	Exon17	Frameshift	-	*De novo*	Pathogenic (PVS1 + PS4 + PM2_Supporting)	[8, 62–66]
10	c.1485_1486del	p.C496Ffs*9	Exon12	Frameshift	EGF_CA	Mother	Pathogenic (PVS1 + PS4_Moderate + PM2_Supporting)	[61, 67–69]
13	chr20:10018942–17,550,915	?	-	Deletion	-	Unknown	Pathogenic (Section1A + 2 A + 3 A + 5 F = 1 > 0.99)	This study
14	c.162del	p.C54Afs*106	Exon2	Frameshift	MNNL	Mother	Pathogenic (PVS1 + PM2_Supporting + PP4)	[59]
16	c.100 C > T	p.Q34*	Exon2	Nonsense	MNNL	*De novo*	Pathogenic	[59]

MNNL – N-terminus of Notch ligand, EGF – epidermal growth factor repeats domain, DSL – Delta serrate ligand, EGF _CA – calcium-binding EGF-like domain, CR –cysteine-rich region, CM-TM – region between CR and TM, TM – transmembrane domain

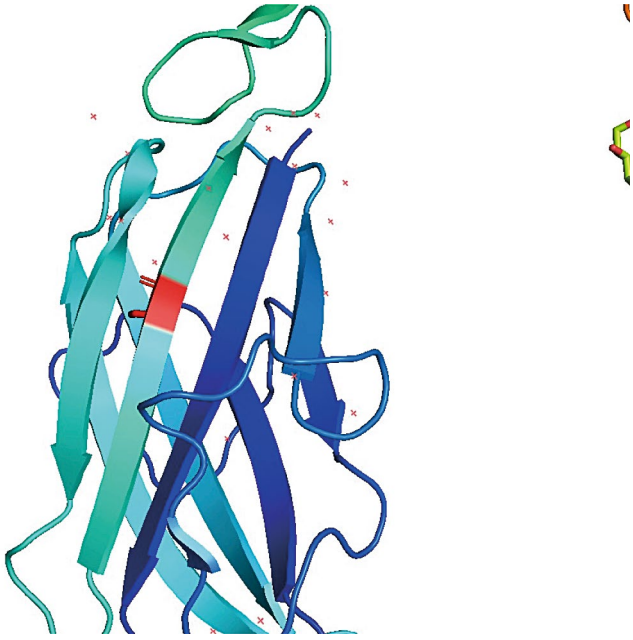

Fig. 4 The protein structure of mutation p.I183N was shown in red (PDBID:4CC0)

Abbreviations

ALGS	Alagille Syndrome
JAG1	Jagged Canonical Notch Ligand 1
NOTCH2	Notch Homolog 2
GGT	Gamma-Glutamyl Transpeptidase
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
hg19	human reference genome version 19
BWA	Burrows-Wheeler Alignment
GATK	Genome Analysis Toolkit
SNPs	Single-Nucleotide Polymorphisms
ANNOVAR	ANNOtation VARiants
SIFT	Sorting Intolerant From Tolerant
SNVs	Single-Nucleotide Variants
ACMG	American College of Medical Genetics and Genomics
VUS	Variants of Unknown Clinical Significance
WES	Whole-Exome Sequencing
PCR	Polymerase Chain Reaction
AST	Aspartate Transaminase
LAP	Leucine Aminopeptidase
TBA	Total Biliary Acid
ALT	Alanine Aminotransferase
TC	Total Cholesterol
TG	Triglycerides
TBil	Total Bilirubin
DBil	Direct Bilirubin
ALP	Alkaline Phosphatase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-04973-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions
MZ, JGY and YZH conceived the idea and conceptualised the study. JGY and YZH collected data.JGY, YZH, ZQX, YD, FCW, YJG, LLC and DNF analyzed the data. JGY and YZH wrote the main manuscript text and repared all figures. MZ reviewed the manuscript. All authors reviewed the manuscript.

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Data availability
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of The Fifth Medical Center of Chinese PLA General Hospital. We obtained written informed consent from all subjects and/or their legal guardian(s) for publication of identifying information/images.

Consent to publish
Informed consent from all subjects and/or their legal guardian(s) for publication of identifying information/images was obtained.

Competing interests
The authors declare no competing interests.

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